

No way to welcome spring

The calculated bad manners towards each other of the two major superpowers may presage better things, as last year, but that is the most optimistic reading of events.

ONLY a few months ago, the general cheerful opinion was that 1986 would be the year in which the wagon of strategic arms control began to roll again. There were several hopeful signs: the generally constructive meeting of the US and Soviet heads of government at Geneva last November, the apparent belief of both leaders that they can gain from arms control (Mr Gorbachev, economically; President Reagan, politically and in reputation), the encouraging noises at the Stockholm conference on European Security over the past several months and the widespread fear that if things do not get better soon, they may get a lot worse. To be sure, nothing much has happened to put flesh on these hopeful bones, but the Soviet Union has offered (and kept) a unilateral moratorium on nuclear testing (due to expire last weekend) and has proposed an ambitious plan for getting rid of strategic nuclear weapons by the end of the century. The chief response so far from Washington has been ambivalent; President Reagan has been lukewarm but has not rejected the proposals outright, and is plainly anxious to fix a date for this year's summit meeting, but other US officials have been downright cool. The British government, responding to the narrow question of whether British nuclear weapons should be restricted at an early stage, has also been downright cool. And now, in the past few weeks, the two superpowers have taken to public rudeness on a scale that will be hard to sustain.

The cheerful reading of these events is that they are a deliberate smokescreen behind which patient diplomats are hammering out the essence of arms control agreements. It is true that the useful meeting last November was preceded by a bout of almost unprecedented recrimination. So too were the months preceding the first of the important agreements on nuclear arms, the partial test-ban treaty of 1963. One of the impediments now to the optimistic view is that the present period also resembles that at the end of 1980 when the Soviet invasion of Afghanistan helped to put paid to the nearly complete draft of a comprehensive test-ban treaty. The danger in the present climate is that the date for this year's planned summit meeting will not be fixed until it is too late for it to be held, with the mid-term elections due in the United States at the beginning of November.

Yet much of the disagreement between the East and the West on arms control consists, in the broadest sense, of misunderstanding. On many of the measures up for agreement, the two sides tend to blow hot and cold, but rarely simultaneously. In 1980, the US administration was keen on a comprehensive test-ban (but may not have been able to win the agreement of the US Senate and its lobbyists); now the shoe is on the other foot, with the Soviet Union being prepared to accept a degree of on-site inspection while the United States, conscious that new missiles will need new warheads and bemused by the Strategic Defense Initiative (SDI), would prefer to talk about almost anything else (except SDI). The essential difficulty is that which has beggared the arms control process from the outset, that of regarding the outstanding problems from the other side's point of view.

Here are some issues on which heads should be banged

together in the next few weeks.

● **Intermediate missiles.** An agreement should be possible this year, especially now that no account will be taken of medium-range aircraft at the outset. One snag is the Soviet determination to keep some mobile SS-20 missiles in Asia; the Soviet Union appears not to appreciate that to require good faith of the United States that these machines would not be moved westwards predicates agreements that have not yet been reached. Another snag is European discontent at the prospect of a European theatre free from all but tactical nuclear weapons and the British and French nuclear forces; but that objection cannot stand in the way of an agreement, which would merely restore the state of affairs before the decision of the North Atlantic Treaty Organization (NATO), in December 1979, to modernize European nuclear forces in response to the appearance of the SS-20s.

● **Strategic missiles.** The Soviet Union and the United States both seem prepared to contemplate substantial reduction of the present limits (those of the unratified SALT II treaty), perhaps by a half. In this context, long-range bombers are bound to matter. A cap on the British and French forces (and the Chinese?) would be appropriate. The United States should appreciate that Soviet unwillingness to carry a larger proportion of its strategic forces on submarines is rational (too many eggs in one kind of basket); the Soviet Union should understand that a substantial reduction is an indispensable prelude to abolition, Mr Gorbachev's goal for 2000.

● **Comprehensive test-ban.** The needs of SDI apart, the United States is at present at a disadvantage in the utility of its missile forces and an agreement on strategic missiles, almost within grasp, will not come about unless US tests continue for some time: the best may be the enemy of the better.

● **Strategic Defense Initiative.** The United States seems not properly to appreciate why the Soviet Union so resents this scheme. Response, either by emulation or the proliferation of existing missile forces, will be ruinously expensive. Even if an effective multi-layered defence against strategic missiles proves feasible, installing such a system over a period of time that must run into decades would be perhaps ruinously dangerous as well. And there is no doubt that the possession of an effective SDI by one side but not the other would be a basis from which to launch a risk-free pre-emptive strike. But the Soviet Union should clarify its reasons for opposing SDI so vociferously. Is the venture fated to be a technical failure, as many critics in the West insist? Might it succeed, in which case why not be prepared to negotiate phased installation? Or will SDI finish up as a mixture of an early warning system and a terminal defence against some missiles (in which case the Anti-Ballistic Missile Treaty would have to be renegotiated)?

None of these arguments is especially profound. No doubt the professional military people on both sides have more specific but more urgent anxieties about the ways in which their opponents are likely to benefit from arms control agreements. The disappointment of the past few months has been that neither side appears to have been ready to acknowledge that anxiety of this kind is both legitimate and unavoidable.



Yet in the absence of such an understanding, agreement will not be possible or, worse, will be reached and then broken.

Another urgent need is for an open recognition that effective strategic arms control is necessarily a slow process. At any time, such as now, it is usually possible to distinguish between the steps that logically come next and those that can only follow. Mr Gorbachev's proposals earlier this year differ from previous versions in their open recognition of this truth. And in spite of the coolness with which some have greeted them, it is plain that they are also negotiable proposals. Why not at least find out, by attempting to negotiate on them? □

Nuclear virulence

Some of the problems of the nuclear industry are illustrated by a book review this week.

NUCLEAR engineers, others who work in the nuclear industry and, indeed, those who use electricity as a fuel, should take care to read the book review by David Pearce, professor of economics at University College London, which appears on page 403 of this issue. To profit from the exercise, it is not necessary to agree with Pearce, or with the author of the book he is reviewing, both of whom are saying that civil nuclear power in Britain and probably elsewhere is a deception practised on an innocent public and on gullible politicians by a bunch of 1950s scientists and technologists ignorant of the imperatives of the social sciences and incompetent as well. The benefit will be the better understanding of the obstacles that will have to be faced by those who would both breathe new life into national nuclear programmes that have fallen on bad times, or who would ensure improved public safety by the better management of nuclear plants.

Do-gooders of that kind should be the first to know what lies ahead of them, to which end Pearce should be generously forgiven his restraint, despite which his piece of action writing is as salutary a warning as there could be against all things nuclear. That the British decision to build advanced gas-cooled reactors in succession to the first generation of natural uranium reactors turned out to be economically unwise is now well-documented, but he does not need, with the passage of so much time, to spare the blushes of those who took the decision, and who are now mostly dead in any case, by concealing the explanation. The decision was technically unsound and was widely said to be so at the time. The problem, in 1960 or thereabouts, was that people were faced with the familiar problem of not knowing how far ahead to jump in the development of a novel technology. The besetting British sin is to jump too far (with the first supersonic civil airliners and so on), but on this occasion they erred in the other direction.

Pearce is also overkind to British nuclear engineers in ascribing the concealment of the truth about nuclear energy to the cover-up engineers who work in public relations and for lobbying organizations. Why not tell it like it was (and, perhaps, still is) that the founders of the British nuclear industry were woefully ignorant of the basic parameters of their craft, the need to anneal irradiated graphite, the corrosion of magnesium by carbon dioxide and the embrittlement of steel by helium gas, all of which they should have learned about at school (together with the biological effects of ingested plutonium)? But his echo of the contention that the problem of waste management must be "solved" before civil nuclear power is exploited cannot of course be gainsaid, although it would have been helpful to have had an economist's view of what constitutes a solution to such a problem.

What Pearce has done is to provide a vivid illustration of the depth of feeling, even passion, that now attends the nuclear issue even in previously sleepy places such as Britain. Many will be grateful to him, especially those who manage without electricity. □

Good luck strikes back

British astronomers should move their surplus observatory to a university.

LONG delayed decisions become palatable because those most affected by them have been anaesthetized while waiting. This is probably why British astronomers appear unperturbed by the announcement last week that the Royal Greenwich Observatory (RGO), founded as an applied science laboratory to exploit seventeenth-century astronomy for better navigation and which left the site whose name it bears nearly forty years ago, is once again to move, perhaps even to merge with the Royal Observatory, Edinburgh. British astronomers are now thoroughly used to the notion that support services for astronomy must be reduced. As one distinguished committee after another has failed to reach a firm conclusion, the exasperated victims have often been chief among those pleading with the executioner to get on with his task.

On this occasion, remarkably, the need that something should be done is not a mark of failure, recklessness or the lack of funds, but if anything the opposite on all three counts. For the best part of fifteen years, or at least ten, the Science and Engineering Research Council (SERC) has dealt imaginatively, responsibly and generously with optical and near-optical astronomy. Capital investment in new telescopes runs close to £50 million, and there is a substantial community of professional astronomers fighting for time on this equipment. One measure of quality of the equipment available to British astronomers is that a SERC committee seriously considered, a year ago, the abandonment of the excellent partnership with Australia in the Anglo-Australian telescope. The two British-based observatories, meanwhile, have found their usefulness diminish. Each of them has provided important support for the building of the new telescopes, but it has been plain for years that there would be no need for both of them when that work was done, as it will be long before 1990.

Two questions arise, only one of which is of a muck-raking character. Most of the decisions about the construction of new telescopes were taken a decade or more ago, when public funds, although already constrained, were easier to come by than at present. In the past few years, the climate has changed so much that it is unthinkable that the same decisions would be taken now; British astronomers are lucky to have squeezed in under the wire in this way.

So why has there been such diffidence in deciding what to do about RGO? And although SERC has laid down that the move of RGO from its present cloudy site must be "self-financing", why is nothing said about the financial savings that may flow from the decision that RGO should move? If there are none, people will be asking whether the move can be worthwhile.

The second question is raised by the incompleteness of last week's decision. RGO is to move, but will be told only in June where and with whom it will spend the rest of its days. SERC seems to be faced with two choices. A merger of the two ground-based observatories would yield economies of scale and concentration, but SERC judges that an institution based in Edinburgh could not have the close links with academic astronomers that would be fruitful for both sides. This is the spirit in which the research council has invited "expressions of interest" (otherwise known as bids) from universities willing to take in RGO as a kind of paying guest. Cambridge and Manchester are the chief contenders. It should be plain to SERC that the benefits of an academic partnership are huge, and that the university option provides British research institutes with a chance to give back to British universities some of the resources and responsibility of which they have been robbed over the decades. But would it not be invidious to pick one out of two? Maybe, but to settle for a merger would be wrong. □

AIDS research

New human retroviruses: One causes AIDS . . .

ACQUIRED immune deficiency syndrome (AIDS) can be caused by a second virus, at least 30 per cent different in sequence from the virus particle, termed HTLV-III in the United States and LAV in France, that is currently implicated in the disease. So claimed Luc Montagnier, head of the French group that discovered LAV at the Institut Pasteur in Paris, at a meeting in Lisbon, Portugal, last week. (HTLV, or human T-lymphotropic virus, was isolated by Robert Gallo and his colleagues at the US National Institutes of Health and Montagnier and his group isolated what they called LAV, or lymphadenopathy virus.)

Montagnier is sending his paper on the

discovery to the *Comptes Rendues* of the French Academy of Sciences, and to *Science*, but was prepared last week to outline what he had said in his Lisbon address. According to Montagnier, the new virus, which he calls LAV-II, was discovered in two patients with AIDS symptoms in a Lisbon hospital. The patients' sera showed no antibodies against LAV (now called LAV-I). There were, however, viruses present that looked just like LAV-I in the electron microscope, and which had the same T-lymphotropic and cytopathic properties.

Nevertheless, DNA probes derived from LAV-I did not hybridize with the DNA from the new virus "in stringent

conditions". Sera from LAV-I patients contained no antibodies for the envelope part of the new virus, although sera from patients infected by the new virus showed a small reactivity with LAV-I core proteins. These data suggested that the new LAV-II virus diverged at least 30 per cent in sequence from LAV-I, Montagnier said.

LAV-II patients also showed some antibodies against the green monkey virus considered similar to LAV-I, "and the interesting question now will be how similar LAV-II is to the simian virus", Montagnier said. **Robert Walgate**

AIDS research

. . . and the other does not

Washington

ANOTHER dispute over scientific primacy has erupted between the United States and France about a human retrovirus. Max Essex and Phyllis Kanki of the Harvard School of Public Health in Boston, together with colleagues at Harvard and in France and Senegal, have found a virus related to Simian T-lymphotropic virus type III found in African Green Monkey (STLV-III_{AGM}) that can be detected in healthy humans. Essex announced the discovery last week to coincide with an announcement by Luc Montagnier of the Institut Pasteur about another human retrovirus (see above). A paper describing Essex's virus will appear in the 11 April issue of *Science*.

Like human T-lymphotropic virus type-III/lymphadenopathy virus (HTLV-III/LAV), the new virus, termed HTLV-IV, infects helper T-cells. But unlike HTLV-III, it is not cytopathic for T-cells.

Last year, Essex and his colleagues reported finding healthy individuals from West Africa who displayed strong antibody reactivity to all STLV-III_{AGM} viral proteins, but reacted weakly with viral proteins from HTLV-III/LAV. They concluded that there was in Africa a human virus more closely related to STLV-III than HTLV-III that either shared a common genetic ancestor or derived directly from the monkey virus.

The Harvard team successfully grew HTLV-IV from serum of three seropositive individuals. Analysis of the new virus shows that it shares many proteins with STLV-III_{AGM}, more so than with

HTLV-III/LAV, notably a 32,000 dalton protein suspected to be a transmembrane envelope protein. The analogous protein in HTLV-III/LAV weighs 42,000 daltons. Electron micrograph morphology of HTLV-IV likewise shows greater similarities to the monkey virus than to HTLV-III/LAV.

At a press conference at the American Society of Microbiologists' annual meeting in Washington, DC, Essex said the new virus described by Montagnier "appears to be related" to HTLV-IV. Unlike LAV-II, HTLV-IV does not appear to cause illness, but Essex would not rule out the possibility that infected individuals might develop disease later in life. Essex believes there is a continuum of retroviruses from those closely related to STLV-III to those closer to HTLV-III/LAV, and it may be that LAV-II lies on that continuum.

Essex was prompted to release details of his discovery in advance of its formal publication because of intense pressure from the media both on him and his collaborator Francis Barin in Tours, France, to comment on Montagnier's discovery. Essex maintains he learned of Montagnier's work only on 22 March, and then only at second hand. Essex worried that he would be "raked over the coals" by the press for refusing to comment on his own work at a time of intense public interest in human retroviruses.

US journalists were alerted to the Institut Pasteur discovery by a press release from a New York public relations company. **Joseph Palca**

NSF tests mini-supercomputer

Washington

THE National Science Foundation's San Diego Supercomputing Center (SDSC) is gearing up for tests of a mini-supercomputer that could save time and money for thousands of Cray users. The SCS-40 or "Crayette" hardware contains the instruction set of a Cray X-MP and can write or run programs compatible with the mammoth number crunchers. When the machine arrives in July, computer scientists will judge whether the \$600,000 crayette can fill the gap between its \$10 million parent and the average patron.

Scientific Computing Systems in California built the SCS-40 in less than two years to provide an intermediate for local Cray programming and debugging, saving Cray time for the largest calculations. With a quarter of a Cray's capacity, the SCS-40 could also expand the computing power of smaller facilities.

John Connolly, head of the supercomputer project at NSF, says that if the SCS-40 makes the grade it could be added to hundreds of remote facilities for the Crays at Carnegie-Mellon University in Pittsburgh and the University of Illinois at Urbana-Champaign, in addition to the San Diego site. Nearly half of the advanced scientific computing budget is eaten away by the leasing of the Crays installed in these centres. Connolly thinks the auxiliary crayettes would prove to be cost-neutral compared to direct Cray use for programming. The SCS-40s might realize their greatest savings in reduced time spent in labour-intensive programming.

Software simulations of Cray architecture have been devised, but are orders of magnitude slower than the Crays themselves and overwhelm lesser machines. Cray's own attempt to build a mini version was shelved in the prototype stage because the market differed from Cray's established clientele. Several other start-up companies that attempted the project could not rally sufficient support. By 1987, the verdict on the SCS-40 should be in. **Karen Wright**

US patents

Doubts on secrecy order

A PROPOSED new secrecy order for controlling militarily sensitive patent applications is starting to worry US high-technology industry. The proposed order, shortly to be published for comment by the US Patent Office, would be used for controlling patent applications containing information that, while not classified, is subject to export control under new authority given to the Department of Defense (DoD). Some fear the new order might make difficulties for US high-technology industries seeking patent protection abroad.

The Patent Office has long had the power to place secrecy orders on patent applications containing information whose publication would harm national security. Two of three new categories of secrecy order now proposed under the 1951 Invention Secrecy Act essentially formalize existing practice. In a nutshell, patent applications are reviewed to determine whether a secrecy order is necessary; if it is, no patent issues while the order is in force, and the inventor is ordered not to disclose the substance of the application to third parties. Patent applications in foreign countries are not permitted while the secrecy order remains in force.

The third new proposed secrecy order, for non-classifiable but export-controlled material, is rather different. It would allow US citizens access to the patent application for legitimate business purposes, and would allow patent applications in foreign countries that have export control rules and reciprocal secrecy agreements with the United States.

Concern that this might make problems for US industry have been raised by the American Association for the Advancement of Science. Although at first reading the new order seems to be a liberalization of existing practice, in fact new authority granted to the Department of Defense in its 1984 authorization

greatly extends its scope to label information sensitive and hence subject to export control. In the past, patent secrecy orders have been used only rarely for inventions arising from privately-funded research. These were the sort of inventions that, had they arisen from government-funded research, would have been instantly classified. But the new "secrecy order and permit for filing in certain countries" could be applied far more often; guidance for classifying technology as export-controllable comes from the militarily sensitive technologies list, which covers a great deal of ground.

A US inventor who found his patent application subject to one of the new orders would be able to manufacture and sell the invention in the United States (this is a "legitimate business purpose") but would risk the invention being copied and "reverse engineered" by competitors overseas while the secrecy order was in force. In the area of electronics, countries on the Pacific rim pose a particular threat. Although the inventor would be confident of ultimate patent protection in the United States and in the other countries named in the secrecy order, by the time a patent issued the market might be flooded with foreign copies. To avoid this, inventors might choose to sit on their inventions until the secrecy order expired and a patent issued, even at the expense of not establishing a captive market.

The Patent Office has said that the new proposals will be published in its official gazette for comment before being brought into force, and believes the congressionally-mandated test of when to impose secrecy orders (possible harm to national security) is unchanged. The Congressional Research Service has raised some doubts about the broad definitions in the order but finds no major legal flaw. Manufacturers' organizations are starting to take notice. **Tim Beardsley**

Mars mission

NASA selects contractors

Washington

PLANS for a Mars Observer mission crept toward completion this week with the selection by the National Aeronautics and Space Administration of contractors for the spacecraft and booster components. NASA named RCA Astro-Electronics to build the spacecraft, which has an estimated price of over \$250 million, while Orbital Sciences Corporation will provide the \$20 million propulsion system. But NASA has already been dealt another delay in the form of a bid protest filed by Hughes Aircraft in February.

NASA's Jet Propulsion Laboratory (JPL) directs the Observer mission, which was proposed in 1983. The first in a series of low-cost inner planet probes, the spacecraft will borrow the design of Earth orbiters and is scheduled for launching from the United States's space shuttle in 1990. By 1991, Observer will be circling Mars for a full martian year. The spoils of the mission may prove useful in organizing the manned mission to Mars which, according to the issue of *Aviation Week and Space Technology* for 24 March 1986, will be suggested by the National Commission on Space early in April.

Hughes's protest stems from a JPL announcement on 20 February that NASA had expressed a preference for an independent upper stage booster design, and that proposals for integrated spacecraft-booster designs would not be considered. With this decision, NASA essentially threw out proposals submitted by Hughes, RCA and Ford Aerospace in mid-1985. But each of these companies was still in the running for the spacecraft contract, having also prepared proposals for the spacecraft alone.

Although Hughes will not comment, one NASA official says it believes it would have won the bid on integrated designs. In a statement issued last week, JPL's director Lewis Allen insists that RCA would have been victor.

A conference on 10 April at the General Accounting Office (GAO) will give both sides a chance to air their views, but GAO may not offer a resolution until June.

In the meantime, NASA cannot award a contract to RCA, and contract negotiations are stalled. JPL's confidence in the outcome, however, can be gauged from the laboratory's activity in the midst of the fervour: JPL has begun distributing RCA's spacecraft specifications to potential bidders for the mission's complement of scientific instruments.

Karen Wright

UK Strategic Defense Initiative contracts

Company	Project	Duration of contract (years)	Amount
Ferranti	Optical computing	1	\$142,500
General Electric	Concept definitions	1	\$100,000
General Electric	Kinetic energy weapons	1	\$100,000
Heriot-Watt University	Optical computing	1	\$142,500
UK Atomic Energy Authority	Neutral particle beam	5	\$10,000,000

ALTHOUGH the agreement signed last December for British participation in the Strategic Defense Initiative (SDI) guaranteed no specific sums, British companies had hoped to garner about \$1,500 million-worth of SDI contracts. So far, at

least, that figure is a long way off. The table above is compiled from figures supplied last week by the Federation of American Scientists. The SDI office in Washington will not confirm or deny these figures. □

US research

Smithsonian center to close

Washington

ALTHOUGH it was known to be coming, the timing of the Smithsonian Institution decision to close the Smithsonian Environmental Research Center (SERC) in Rockville, Maryland, later this year has prompted cries of outrage from SERC-Rockville employees and their supporters.

Notice of the decision was contained in a memorandum sent to SERC-Rockville director William Klein on 21 March by Smithsonian assistant secretary for science David Challinor. During a visit to SERC-Rockville last February, Challinor told Klein and others that the Smithsonian was thinking of closing the center during the 1987 fiscal year. The March memorandum stated that the facility would close and all positions would be abolished on 14 November 1986, six weeks after the start of the 1987 fiscal year.

While the Smithsonian maintains that some employees will be offered positions at other Smithsonian facilities, that is not the impression left with employees.

What particularly rankles with people at SERC-Rockville is that they feel deci-

sions were made without their involvement. "I have not been consulted about this decision whatsoever", says Klein.

Also annoying has been the Smithsonian's opinion that SERC-Rockville's productivity "was not up to the standards of similar institutions". Those familiar with SERC-Rockville's work concede that the facility could have helped its cause by publishing more frequently.

SERC began in 1929 as part of the Smithsonian Astrophysical Observatory and became an independent bureau in 1969. It now occupies a leased facility in Rockville, a suburb of Washington, DC. The staff consists of 11 scientists and 34 support personnel, with an annual budget of around \$2 million. The centre is primarily devoted to photobiology, with current research projects focusing on blue and red light photoreceptors, accessory pigments and CO₂ photosynthesis. In addition, there is a solar radiation monitoring group and a carbon-dating facility, both likely to be retained elsewhere by the Smithsonian.

Two independent reviews of SERC-Rockville conducted in 1979 and 1981 for the Smithsonian concluded that the labo-

ratory lacked the "critical mass" of people and facilities to perform world class science. The possibility was considered of merging SERC-Rockville with a smaller SERC facility on the Chesapeake Bay, but the \$30 million price tag for a new facility was beyond the institution's budgetary grasp.

Several universities, including Johns Hopkins, Duke and the University of Maryland, expressed an interest in merging with SERC-Rockville, but were unwilling to make the financial commitment that the Smithsonian wanted.

The Smithsonian Institution ultimately decided that it could not afford to support SERC-Rockville at a level it felt was necessary for good science, and the decision to close the centre was hastened by the fact that the lease on the Rockville facility was due to expire in 1990.

Winslow Briggs, head of the department of plant biology at the Carnegie Institution of Washington in Stanford, California, calls SERC-Rockville's work "not terribly fashionable, but terribly important". He expressed disappointment at the Smithsonian's decision, and "outrage" that the closure would take place this year.

The Smithsonian is not blaming budget problems as the primary reason for the closure. Instead, says Ross Simons, programme manager in Challinor's office, the issue is "where Smithsonian science as a whole should be going". Simons says the Smithsonian's scientific strength in the natural sciences is in evolutionary biology, and that is where money saved from the SERC-Rockville closure will be spent. **Joseph Palca**

US engineering

Five more centres named

Washington

THE National Science Foundation (NSF) last week announced five more Engineering Research Centers, intended to foster interdisciplinary research and education in selected engineering fields. The five new centres, selected from 102 proposals from 75 institutions, will together receive \$56,300,000 over the next five years.

The first six NSF engineering research centres were announced in May 1985 in response to a widespread sentiment that NSF should be doing more to foster engineering research. Since then, NSF authorizing statutes have been amended to give greater prominence to engineering. But even with the new emphasis (enthusiastically championed by NSF's director Erich Bloch) the number of centres will not reach 25 by the end of fiscal

year 1987, a previously discussed target.

The existing six centres have succeeded in attracting substantial industrial support: \$13 million on top of the original \$10 million from NSF. The newly announced centres are expected to do likewise. Typically, 20-40 faculty members are involved in a centre, and collaboration with industry on engineering problems is mandatory. Observers are encouraged by progress so far. According to Lynn Preston of NSF's cross-disciplinary research directorate, the centres have inspired other universities and industry to establish cooperative research links along NSF lines even without NSF support. Some similar collaborative research centres are proposed to be funded this year by the Department of Defense under its new University Research Initiative. **Tim Beardsley**

Institution	Subject	Amount (over 5 years) (\$ million)
University of Utah & Brigham Young University	Advanced combustion Engineering	9.7
Carnegie-Mellon University	Engineering design technology	14.9
University of Illinois-Urbana	Compound semiconductor microelectronics	11.6
Lehigh University	Large structural systems	10.4
Ohio State University	Net shape manufacturing (direct near-final shape production)	9.7

EPA bars AGS test

Washington

THE Environmental Protection Agency (EPA) has suspended the experimental use permit issued to Advanced Genetic Sciences (AGS) for field trials of genetically altered bacteria on strawberry plants until new data can be reviewed.

EPA will also fine AGS \$20,000, the maximum allowed by law, for testing the genetically altered version of *Pseudomonas syringae* under conditions that could have led to an environmental release of the bacteria. The EPA complaint charges AGS with misrepresentation and falsification of data, as well as use of an experimental substance in a manner not prescribed by agreed protocols.

Last month, AGS admitted that pathogenicity tests on the new bacteria, developed to protect crops from frost damage, were carried out on trees on the roof of its Oakland headquarters (see *Nature* 320, 2; 1986). EPA regulations require such tests to be conducted in an enclosed environment. AGS has now agreed to redo pathogenicity tests on the trees. **Joseph Palca**

French science

Bureaucracy looks to survival

IN an extraordinary declaration of the likely directions of the new French government's education policy last week, the new minister of education, René Monory, has announced that he will make no radical alterations to the constructive policy of his socialist predecessor, Jean-Pierre Chevènement.

Monory told journalists that he "wanted to get things moving" and that he agreed with most of the things that Chevènement had been doing. He was particularly happy, he said, with Chevènement's efforts to raise the number of schoolchildren achieving the "baccalauréat" (the high school graduation certificate that guarantees entry to university). "International competition and demography demand it", he said.

Thus Monory has pinned his colours to Chevènement's typically radical efforts to reform the school system, which for Chevènement was too geared toward mathematics and to the entry of the very few into a few élite institutions.

Chevènement's intention was to create new universities of technology and more *grandes écoles* geared to tomorrow's technology. But pre-election advisers to the new Prime Minister, Jacques Chirac, sought to strengthen and return power to the existing university

system, and had been working on legislation to this end. Chevènement's grand plan will not now be torn up. Chirac and Monory may be planning to go easy; Monory is talking of not wishing to "rekindle the battles", said Monory.

The government's policy remains far from clear. Bernard Descompes, who masterminded the previous government's successful attempts to tighten up assessment and research quality in the French universities, was due to meet his new minister for higher education and research, Alain Devaquet, just before the Easter break. Whether the old ministry of research and technology will survive is apparently not yet decided.

Meanwhile, Devaquet occupies not the small office at the ministry of education appropriate to a junior minister but the grand headquarters of the old ministry of research and technology, in the old École Polytechnique on the Montaigne St Genevieve. The switchboard operators are selfconsciously announcing "ministry of higher education and research" to incoming callers. Some on the Montaigne hope that the ministry will not be as radically dismantled as Chirac has been threatening.

Devaquet has also filled the powerful post of cabinet director with Michèle

Legras, who is four years older than her minister and a career administrator of some eminence; she has served under ex-prime minister Raymond Barre and was *chef du cabinet* to a previous (but regrettably forgotten) research minister, Jacques Sourdis, in 1977.

Robert Walgate

Polish nuclear power

Safety fears

LOCAL opinion in the district of Lubusz in western Poland is "outraged" by plans to build a nuclear waste dump, according to Zielona Gora local radio. Members of the Sejm (parliament) are accordingly being sent to the area to confer with local officials, although no final decision on where the waste will be stored can be reached until an expert survey is completed in 1990.

At present, a new bill on the nuclear industry is being drafted. Polish media and press agency reports on the bill, during the past two weeks, have stressed that it will make all necessary provisions to protect workers in the nuclear industry and the public at large from radiation hazard. This stress on safety is clearly meant to allay fears not only in Lubusz but also in the vicinity of the planned nuclear power stations at Zarnowiec and Włocławek.

The fears go beyond the usual apprehension of residents of an area where a nuclear installation is planned. Nuclear safety in Poland is by no means well organized, but is at present governed by a regulation dating from 1961 which involves not only the Atomic Energy Commission but four other ministries as well.

In 1973, the second congress of Polish science called for a two to threefold increase in the number of nuclear safety experts, but numbers have instead fallen rapidly (by 45 per cent between 1980 and 1985), while those who remain are mostly approaching retirement age. In May 1985, the academic council of the Institute of Nuclear Chemistry and Technology at Zeran, stated that Polish radiological protection work is in so disastrous a state that its very existence is threatened.

How far the new bill can remedy this situation is unclear. The main need seems to be the training of a new generation of safety experts, which will take several years. But the rapid response of the Sejm to the disquiet in Lubusz, to say nothing of the fact that it was reported in the official media, suggests that the Polish authorities, now irrevocably committed to a programme of nuclear expansion, are anxious that fears over safety should not escalate into the kind of anti-nuclear protest now building up in Yugoslavia.

Vera Rich

Aleksandrov stays at Soviet academy

ACADEMICIAN Anatolii Aleksandrov, the 83-year-old president of the Soviet Academy of Sciences, has retained his post, in spite of rumours that he was to resign at the annual general meeting on 19 March. Most rumours tipped as his successor Academician Evgenii Velikhov, a physicist who has recently entered the arena of international politics as head of "Scientists against Nuclear War". Shortly before the meeting, however, Academician Viktor Logonov, the rector of Moscow University and also a physicist, was also mentioned as a possible successor. In the event, in spite of Mr Gorbachev's current policy of retiring eminent but superannuated people, Aleksandrov was not replaced.

Aleksandrov is not in the best of health, and has not for some years attended ceremonial functions abroad. His continuation in office will mean continued diplomatic limelight for the vice-presidents, who deputize for him, which may not be entirely unwelcome to those not eligible for the presidency. Over the past few years, it has become a tradition that the president of the academy is either a mathematician or a physicist.

Apart from Aleksandrov's staying on, this year's meeting brought few surprises. Most speeches dealt with the implementation of the resolutions of the recent party congress, insofar as they affect science.

Aleksandrov himself noted that the role of the academy as a coordinator of all research work in the Soviet Union should be "appreciably enhanced" in the new five-year plan (1986-90), gave the number of researchers in the Soviet Union as around 1.5 million and noted that this year's research budget (presumably for the whole of the Soviet Union) would amount to 29,000 million rubles. Other speakers dealt with the priority areas already outlined in the guidelines for the plan, but Academician Yurii Ovchinnikov complained that although genetic engineering can produce new medicines in six months, the time needed to test and "verify" them (10-12 years) was much too long.

One innovation that seems to have been generated within the academy is the decision to create an interdisciplinary coordination council for the investigation of the "entire package of questions of the study of man".

Vera Rich

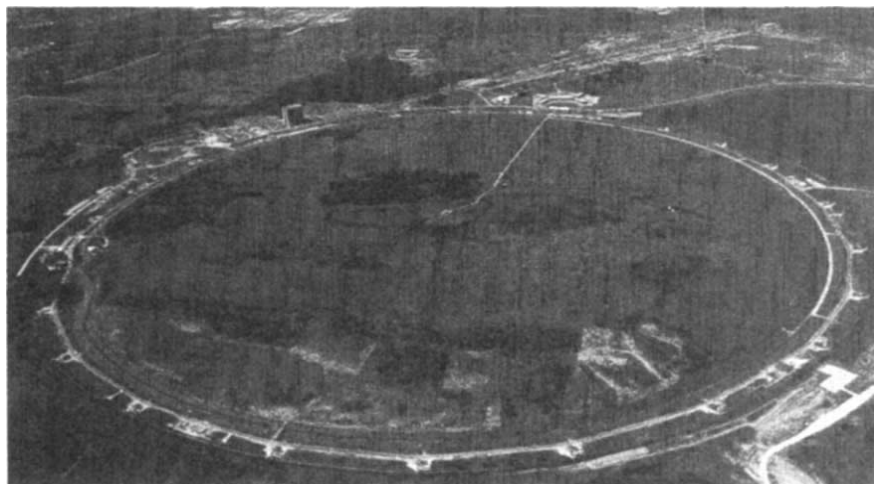
Fermilab

High-energy physics in a suitcase

Batavia, Illinois

PHYSICISTS at Fermi National Accelerator Laboratory (Fermilab) are not the only ones disappointed that the main accelerator beam was shut down for repairs after last fall's successful engineering run that produced the highest energy

Most visiting scientists, called "users" by the resident staff, stay at the Fermilab village, once the town of Weston, Illinois. Collaborators on Fermilab projects who still have teaching responsibilities at their home institutions face an itinerant lifestyle. "I have my bags



proton-antiproton collisions ever. A flock of Canada geese typically spends the cold Illinois winter on one of the cooling ponds for main ring magnets. With the ring's magnets turned off, the ponds are now mostly ice, so that this winter the large flock has had to fight for space in the small area melted by heat from Fermilab's computers.

Fermilab sits on 6,800 acres about 30 miles from downtown Chicago. Despite its relative proximity to one of the largest cities in the United States, a herd of 75 buffalo grazing just beyond the Collider Detection Facility (CDF) serves as a reminder that the city is not that close. Indeed, a conscious effort has been made to minimize the environmental impact of Fermilab's facilities. A project begun nearly a decade ago by Fermilab employees and area residents has now replanted all 800 acres inside the ring, restoring much of the original prairie vegetation.

Fermilab was dedicated in 1974. Today, there are about 2,200 employees, although that number will be allowed to slip to 2,000 under budgetary pressures. There are 400 physicists resident at Fermilab, but there is also a steady stream of visiting physicists from the 325 institutions in the United States and abroad involved in collaborative projects.

Fermilab budget (\$ million)

	FY85	FY86	FY87 (estimate)
Operations	122.3	118.8	135.9
Capital equipment	27.5	31.6	30.5
Construction	41.6	19.3	23.7

packed all the time", says Phil Kesten, a postdoctoral fellow at Brandeis University working on software for CDF. Kesten leaves his home in Boston once every 2 to 3 weeks for four-day visits to Fermilab. Some pay even more frequent visits.

While the trip to Chicago takes about an hour by car, many are reluctant to spend the extra travel time. The neighbouring towns of Batavia, Aurora and St Charles are hardly famous for their cultural appeal. "One likes to go out to a nice restaurant once in a while", says Kesten. "After a few trips, you've tried them all".

One bonus from living at Fermilab is the opportunity to visit Chez Leon, a restaurant run by Tita Jensen, wife of physicist Hans Jensen. Chez Leon is open only for lunch on Wednesdays and dinners on Thursdays, but visitors have been known to adjust their schedules to coincide with those times.

Users are not alone in being faced with heavy travel schedules. During this period of tight federal budgets, Fermilab project directors constantly find themselves travelling to Washington to defend their fiscal needs. Two weeks ago, Peter Koehler and Paul Grannis, co-leaders of a new colliding beam detector known as D-Zero, made the pilgrimage to urge Department of Energy officials to release an additional \$1 million to support research and development efforts by the 125 collaborating institutions. While Koehler and Grannis are satisfied with the \$7.1 million allocation in the 1986 budget for D-Zero, they are concerned

that without additional money for their collaborators, work will fall behind schedule. But prospects for this extra money are not good. The Department of Energy has already used up most of its discretionary funds for this fiscal year.

Fermilab director Leon Lederman was also in Washington recently, testifying before a House subcommittee on high-energy physics. Reagan administration budgets have treated Fermilab fairly generously. With the commissioning of the Polarized Proton Beam and Target System, expected within five months, Fermilab will finish a \$25 million upgrade to the fixed beam experiments.

The completion of the major Tevatron construction projects brought Fermilab's budget down to \$169.7 million in 1986 from \$191.4 million in 1985. The government has proposed a 1987 budget of \$190.1 million that will allow improvements to the antiproton Debuncher and Accumulator rings, as well as completion of CDF and continued work at D-Zero.

But Lederman believes that science in general is "trembling at the edge of a decline" like that of 1968. Lederman is especially concerned about the effects of the automatic cuts that may be required by the Gramm-Rudman deficit reduction act. "It will kill American science", says Lederman. He argues that most scientific efforts operate on a pattern of growth, and to apply the brakes suddenly can wreak havoc. Moreover, in a large facility such as Fermilab, many capital



Leon Lederman

costs are fixed, and a 15 per cent cut in the overall budget can mean a 40 per cent cut in funds available for research.

For the time being, Lederman believes that Fermilab is "sitting pretty". Until the Superconducting Supercollider is built, and Lederman believes it will be, Fermilab will provide the highest energy window into the particle physics world.

Department of Energy officials concede it will be difficult to obtain the funds budgeted for Fermilab in 1987. But the prospect of losing funds for research is extremely frustrating for high-energy physicists who feel their subject is "incredibly juicy" right now. "It's like being in an attic full of treasures", says Lederman, "and we've opened only one box".

Joseph Palca

West German universities

SIR—With nearly six years experience of the present “democratic” system in one West German university and after working for 10 years in a US university (JVB) and 28 years in a UK university (GE), we believe that Stumpfl (*Nature* 319, 256; 1986) overstates its deficiencies.

The “democratic” committees (on which professors constitute only 50 per cent of the membership, even for the appointment of new professors) are, as he claims, immensely time-consuming and inefficient. However, they provide an opportunity, perhaps the only one, for students to counter the all-pervading attitude that the university exists primarily to support the administration or the teachers rather than to educate the students. It is the rule rather than the exception that professors allow only one hour a week for individual contact with students (the weekly *Sprechstunde*); and there is frequently also little informal contact between professors within one faculty or with the lecturers, there being no equivalent to the regular “lunchtime get-together” or the “common room”.

In our experience, the committees are not “...dominated by extreme left-wing activists”. The real power lies with one individual in the faculty, the dean and his tiny committee, the *Dekanat*. He is responsible for all administrative duties (for example the teaching programme, financial expenditure, the supervision of the lecturers, the allocation of space, arranging for the appointment of new professors, safety, appeals against examination procedures or results).

Since these duties are so arduous (and incompatible with full-time teaching or research), the office is generally held for only one or two years — hardly long enough to learn how to perform the duties competently. Being ultimately responsible but insufficiently experienced, the dean may indeed adopt “populist measures” or have to “bow to the majority of the less qualified” to obtain committee decisions required to keep the faculty running.

Meanwhile, each professor is explicitly expected by all those who work with/under him to advocate lines of action that will afford maximal gain to his particular section of the faculty; hence, self-interested pleading rather than consideration of the overall good characterizes the interminable committee discussion; and the dean, who may not himself be entirely disinterested, lacks the experience to hold out against merely clever arguing.

On this analysis, it is not the “democratic” system in itself that is at fault. Rather, the purposes of the university have been lost sight of (partly due to the sheer weight of the administrative paper

work); and the day-to-day administration falls to those who do not remain in office long enough to learn to withstand those who push hardest.

JOSEPHINE V. BROWN
GEORGE ETTLINGER

*University of Bielefeld,
4800 Bielefeld 1, FRG*

South Africa

SIR—Would it be too much to hope for a cool and rational discussion of the issues raised by the banning of South Africans from the World Archaeological Congress? While there is indeed an issue of principle involved, as Bender *et al.* insist (*Nature* 319, 532; 1986), the decision about what action to take should be made with a view to what is most likely to be effective.

It is presumably this which lies behind the idea of “historical moments” in the lives of repressive governments, and makes it worth serious consideration. However, it also undermines the attempt of Bender and his colleagues to treat South Africa as uniquely awful because it institutionalizes apartheid. Mandelstam *et al.* (*Nature* 319, 715; 1986) are right to condemn them for failing to address the real issue. But they go on to ridicule the use of the “historical moments” argument against South Africa on the grounds that logically we should apply it to many other regimes as well. Although some of the examples they cite are strange — the only “mass slaughters that take place constantly in the Middle East” that I know of are part of the Iran–Iraq war, and they seem unaware of the recent change of government in Uganda — there is every reason for being prepared to treat other repressive regimes in the same way as South Africa’s.

There is no doubt that the attitude to South Africa of the left in Britain has long been distorted by hypocritical double standards. South Africa is very far from being the nastiest country in the world, or even in Africa.

That said, there is a better *prima facie* argument for singling out South Africa at this particular moment than is acknowledged by Mandelstam *et al.* It does have democratic structures and institutions of a kind, and, *pace* Dr Slabbert, a legitimate opposition, and is thus more likely than, say, Paraguay or Syria to respond to boycotts and bans because, unlike them, it has a body politic capable of feeling the pressure.

We should forget the shoddy behaviour and dubious motives characteristic of so many of apartheid’s foreign opponents among the fashionable left, and instead concentrate on what is most likely to bring

about desirable change in South Africa, and in the much more vicious regimes to be found in almost every continent.

A. W. ANDERSON

*University of Oxford,
Department of Biological Anthropology,
58 Banbury Road,
Oxford OX2 6QS, UK*

SIR—The recent letter from Bender *et al.* (*Nature* 319, 532; 1986) supporting the Southampton boycott of South African archaeologists is basically a purely political document, apparently expressing the view that the Botha government will tremble in its shoes when enlightened academics stamp their feet. If only this proposition were true. The arguments presented appear to place little value on either scientific or educational considerations. However as the letter comes from a university address, one must presume its writers are educationists, and so should be willing to state their position clearly as regards educational issues.

As a pragmatic scientist and university teacher, it seems to me their position as educationists must be one of the following:

- (1) It is wrong to provide good quality universities in South Africa. If this is the stance, its basis presumably is that black people do not need a university education.
- (2) It is right to provide good quality universities in South Africa, but those who do so should be penalized for their efforts. This stance seems to be straightforwardly contradictory.
- (3) It is all right to provide undergraduate education in South Africa, but post-graduate education and research should not take place there. If this is the stance, it presumably means that the appropriate ceiling for black people is an undergraduate degree — which is just a sophisticated version of Dr Verwoerd’s vision that they should be educated so far and no further.

- (4) Both undergraduate and graduate university education should be available to blacks in South Africa, but not to whites. This stance is both racist and eminently impractical in terms of educational realities. If this is indeed the stand taken, who will do the teaching, and where will the necessary reservoir of high-level academics come from?

In view of the destructive nature of their stance, your correspondents have an obligation to make their position clear. I should very much like to know which of these alternatives they support (or what other view they have of the education that should be available to the populace of this country).

GEORGE F. R. ELLIS

*Department of Applied Mathematics,
University of Cape Town,
Rondebosch, Cape 7700,
South Africa*

Infectious AIDS

SIR—The letter from the Drs Fox (*Nature* 319, 8; 1986), and your leading article (319, 9; 1986), highlight the recent awareness that the virus known as LAV, HTLV-III, or ARV (hereafter called the AIDS (acquired immune deficiency syndrome) virus) often causes progressive encephalopathy, similar to that in the archetypal slow virus disease of sheep, maedi-visna. However, the generally accepted hypothesis that "the AIDS virus is plainly not particularly infectious" needs to be modified slightly. Under special circumstances the virus is highly infectious.

Infected people are persistently viraemic, and they intermittently shed infected lymphocytes in saliva, semen, bronchial secretions and tears. Serum contains up to 25,000 virions per ml (ref. 1), but virus is largely cell-associated in the other fluids, making them much less infectious than serum. However, AIDS virions remain highly infectious after seven days in water at room temperature, and retain some infectivity when dry for a week². A few AIDS virions injected hypodermically into chimpanzees invariably infects them, and within two weeks their serum becomes persistently infectious.

It is consequently unsurprising that the virus is spread rapidly by repeatedly re-used unsterilized hypodermics, and by sexual manoeuvres that damage the rectal mucosa of people who frequently change partners. Modern medical hypodermics are re-used in poor countries on a very large scale. For example, in four weeks in 1976, the blood-borne virus causing Ebola fever swept through the 120-bed mission hospital in Yambuku, Zaire, because only five needles and five syringes were used each day for all ward patients, and about 400 out-patients³. Injection was the preferred route for all medication. A pan of water was used to rinse the hypodermics, which were boiled less than once a day.

With similar practices widespread in Africa, Asia and South America, millions could be infected with the AIDS virus, brought to a continent by a single carrier, before anyone realized that it had arrived. The incubation period to illness lasts years, and early cases are lost amongst the diseases of abject poverty.

Once a critical mass of people have been infected rapidly by highly efficient means of transmitting the virus, then transmission by far less efficient means will inevitably occur increasingly often. These include blood transfusions, perinatal transmission, biologically normal sexual intercourse, needle-stick injuries, chance contact of sores or abrasions with contaminated blood, saliva or sputum, mechanical transmission by blood-sucking insects and flies and routine dental procedures. AIDS patients who have no known "risk factor"

form the third largest "risk group" for AIDS in the United States (6 per cent), the second largest group in Western Europe and the vast majority in Africa. Postulating clandestine homosexuality or heterosexual promiscuity to explain these cases is unnecessarily speculative, given the properties of the virus.

You mention some of the many similarities between the viruses causing AIDS and maedi-visna. If the long-term mortality of infection also turns out to be similar, the AIDS epidemic is more than "a serious problem of public health"; it is the start of a pandemic slow virus disease with the potential to decimate mankind within a couple of decades.

JOHN SEALE

*Royal Society of Medicine,
1 Wimpole Street, London W1M 8AE, UK*

1. Levy, J.A. *et al.* *Ann. intern. Med.* **103**, 694-699 (1985).
2. Barre-Sinoussi, F., Nugeyre, M.T. & Chermann, J.C. *Lancet* **ii**, 721-722 (1985).
3. Report of International Commission *Bull. Wld Hlth Org.* **56**, 271-293 (1978).

Complex forests

SIR—The erroneous idea is becoming widespread that the remarkable forests being destroyed and in danger of extinction round the tropics, subtropics and in a few temperate lands are rain forests, whereas they are often dry sclerophyll woods where the dead leaves crackle underfoot. The essential thing about them is not wetness; it is that they are all coherent communities which are complex multiple aggregations containing many quite different kinds of trees of all shapes and sizes dominated by lofty, often huge trees many centuries old, each bearing a large quantity of timber. Numerous different species of lianes, shrubs and flowering plants hang on the branches adorning the complexity.

The roof of this kind of standing forest is a billowing surface of greatly varied height and shape, frequently overtopped by the crowns of the scattered dominants. Such a kaleidoscopic vegetation takes many centuries to mature, millennia even, and can never be replanted once it is gone. It is surprising that these ancient complex forests have kept the same habits of growth, shape and structure in so many different countries, each with its own unique combination of species. The soil beneath is often poor and exhausted, and after the big trees are cut, the local microclimate changes, and if the surface does not erode and disappear, nothing but weedy scrub will cover its wounds.

Real rain forests blanket many square miles of the Southern Hemisphere among mountains where precipitation rates may reach 450 cm a year, where there is no dry season and everything is sodden. *Nothofagus* trees, all one species in any one place,

grow laced together to a uniform low to medium height, their twisted branches and exposed roots clad in a thick bryophytic wrapping. Many ferns, saplings, shrubs, seedlings and a few flowering plants grace the interior. Such rain forests form a smooth green surface spreading over the country like a carpet, in strong contrast to the bubbling canopies of a complex forest.

These two distinctly structured types of forest, both of which may cover a range of climatic conditions and may abut without ever mixing, are better called "complex" and "blanket". Complex forests are always ancient and if destroyed cannot be reconstituted. Blanket forests in contrast, with a single tree species in any one area, are comparatively short-lived and may generate readily.

GRETA STEVENSON

*Flat 3, Lockwood Court,
76 Westwood Road, Southampton, UK*

Nature exploiters

SIR—Lord Ashby, in his review of the book *Pesticides and Nature Conservation* (*Nature* 318, 21; 1985), concludes that "there has to be a compromise between those who want to exploit the environment for profit (either by growing crops or making pesticides) and those who want to protect it". He has, however, reversed the roles of the participants.

Those who have become bloated by exploiting the environment for profit are groups such as the Audubon Society, the Sierra Club and the Environmental Defense Fund. They have attained great political clout, huge staffs and bulky portfolios of stocks and bonds financed by the donations of frightened, misinformed citizens who were exposed to the false allegations of pseudoenvironmentalists. Humanity should be included as a part of the environment, and "those who want to protect it" are thus the groups involved in growing crops and preventing illness and death resulting from insect-borne pathogens.

Lord Ashby categorizes John Sheail's book as "a record of missionary work by scientists" who have worked and lobbied on behalf of the environmental movement. Those "missionaries" paid scant heed to the hundreds of millions of human beings who were sacrificed as a result of their efforts, and it might be asked why they resorted to such unscientific methods as deliberately distorting or omitting all the data that refuted their allegations over, for example, the impact of DDT. Lord Ashby is certainly correct: "the story is not over yet".

J. GORDON EDWARDS

*Department of Biological Sciences,
San Jose State University,
One Washington Square,
San Jose, California 95192-0100, USA*

Japanese psychiatry

SIR—As the other visiting scientist named in the article by Alun Anderson entitled "Abuse for visiting scientists" (*Nature* 315, 361; 1985), I would like to add to Kimio Moriyama's response (*Nature* 318, 308; 1985). Dr Moriyama writes that his group did not "intend to visit the smaller meeting at Nagoya". At the smaller meeting, Dr Crow and I were the only two speakers to be scheduled and our presentations were cancelled because the group of psychiatrists and their followers from Tokyo and Gifu were on their way to disrupt the meeting. Previous encounters had led to violent fights between the two groups. I fully agree with Dr Crow (*Nature* 319, 172; 1986). I too am willing to have the abstracts of the cancelled meeting of the Japanese Biological Psychiatric Society, deemed by Dr Moriyama to be unethical, examined by an official Institutional Review Board. I hope that the Japanese psychiatric patients, abandoned by their families, will not be deprived of participation in psychiatric research.

There are apparently some changes now to the betterment of patient rights and patient care in Japan. Changes like that are always too slow. Violent threats and intimidation belong to another era. I do not believe that anyone is served when psychiatrists go into fist fights to disrupt meetings. It will do nothing to remove the stigma attached to the mentally ill or to increase public support for reform. Presumably, we are all involved, because we want to improve our patients' lot and believe in the principle of freedom of scientific information.

DANIEL P. VAN KAMMEN
Veterans Administration Medical Center,
Highland Drive,
Pittsburgh, Pennsylvania 15206, USA

Wasting assets

SIR—I recently attended a one-day international meeting concerned with a fundamental aspect of physics. For twenty-four hours I was free to do my proper job of thinking about science and how mankind can better understand the sensible universe. Such is the contrast between this pleasant and productive interlude and what my life otherwise has become that I actually felt guilty at absenting myself from the usual Sisyphean labour of trying to overcome inadequacy of resources, digging away at piles of unproductive paperwork, making meaningless returns and providing unvalidated "indicators" of performance. It was like attending a party in the penthouse of a building while the foundations are crumbling.

A number of overseas colleagues expressed disquiet at the crisis in funding of British science and education, and even more at the crisis in morale resulting from

continual insults and deprecation, and our despair that the nature and importance of what we do will ever be understood. It became apparent that we have come to mistrust our politicians far more than do scientists of other countries. As a transatlantic colleague put it, while US politicians do indeed devote much attention to the need to be re-elected every few years, they are sophisticated enough to understand that pure knowledge and research are the seed-corn of what the culture and industry of their country will need in ten to thirty years' time.

By contrast, we encounter penny-pinching which is squandering a great national asset, once esteemed as among the finest in the world. The whole structure of our institutions concerned with knowledge, from schools through universities to original research, is in disarray. One can hardly doubt that if present policies continue, then even apart from explicit support for research, we shall lack the structures that enable talented people to be educated and become the creative researchers of the future.

In the absence of the new ideas generated by research, we shall lack an essential ingredient needed for industrial prosperity in future decades. This situation has within it the seeds of Britain becoming a third world country, taking its ideas and industrial capital from more developed countries.

The question is not whether we can afford what we spend on schools, universities and research, but how a densely populated island can afford to spend so little on cultivating its intellectual assets. The knowledge base of our culture and industry is in jeopardy so long as we invest less on it than we wager on horses.

PETER FELLGETT
University of Reading,
Department of Cybernetics,
3 Earley Gate, Whiteknights,
Reading RG6 2AL, UK

Creationism

SIR—We have witnessed, again, the basic underlying view of creationism in D.H. Koobs' letter (*Nature* 319, 172; 1986), where he shows four of the methods creationists commonly use during their assaults on science.

First it is claimed that science does not have a complete understanding (which it never can) of important items such as the origin of life or the Universe, that these are some sort of boundaries to reality, that they are impermeable to human scrutiny and that since they are boundaries not crossable by science they are therefore, by default, proof of their presupposed supernatural creator. Nothing is potentially beyond science.

Secondly, Koobs brings science down to the creationists' level by making the

absurd statement that there are areas of science that are to be taken on faith. I have heard many creationists claim it is a greater leap of faith to believe in evolution than it is to believe in creation. These people use that word incorrectly. Faith is a belief without evidence, and in the case of creationism it is a belief in spite of evidence. However, we all know that science accepts nothing on faith.

Thirdly, Koobs gives the impression that reality is manifested by human emotions and desires with this statement: "... who are free to choose which history provides more meaning for life". No comment is necessary.

Finally, misrepresentation runs rampant in creationism. Koobs is obviously referring to punctuated equilibrium when he claims that animals preserved in the fossil record "occurred spontaneously" and trying to equate punctuated equilibrium and spontaneous generation as acts of creation.

Creationism and science will never be two different ways of looking at reality. Creationists do their best to twist, lie, fabricate, misrepresent all they can of reality to deceive their followers and the lay public.

J. RICHARD WAKEFIELD
385 Main Street,
Beaverton, Ontario, Canada L0K 1A0

Animal deception?

SIR—Several times recently I have read claims (most recently in *Nature* 319, 143–145; 1986) that animals practise deception. There appears to be a strange logic at work here.

First, we have a Cartesian animal, with a limited repertoire of behaviours. There is one behaviour called "alarm call", which the investigator has presumably defined very carefully by extensive observation.

Next, it is reported that the "alarm call" has been used for some other purpose. Instead of concluding that his initial definition of "alarm call" was too narrow, the investigator summons up another animal, one which uses the "alarm call" deceptively. This second, non-Cartesian animal is thus implicitly given credit, or blame, for an act of choice, which invokes a presumption of self-consciousness, even an ethical being.

Ruppell (the author's first citation) may have started this fad with his account of the "lying" mother fox. I believe the investigator is deceiving the investigator here. At the very least, words like "deception", with strong ethical connotations, seem out of place in this sort of context.

HERBERT MCARTHUR
Research Foundation of
State University of New York,
State University Plaza,
Albany, New York 12246, USA

Icosahedral frustrations ahead

The experimenters have been more successful in finding new examples of icosahedral symmetry than the theoreticians have been at interpreting these exciting data.

In the two years since the discovery of an alloy of manganese and aluminium with icosahedral symmetry by Schechtman, Blech, Gratias and Cahn, most of us have become more knowledgeable about crystallography, but are still perplexed to know what icosahedral symmetry means. This is not to suggest that people have been idle. Making tiny samples of alloys other than the Schechtman alloy, but with the same unexpected symmetry, has been widely practised; electron and X-ray diffraction patterns with five- or tenfold symmetry have become familiar.

People with a feeling for solid geometry have become even more expert at telling how icosahedra may be assembled into the shapes called tricontrahedra, while people like Pauling, with a sixth sense for how crystals are constructed, say the ground is familiar. [Pauling's first statement of this case (*Nature* 317, 512; 1985), which has been disputed, will soon be followed by an elaboration.]

The difficulty remains that ordinary mortals have no way of visualizing the construction of an icosahedral crystal, called a quasicrystal because it must be aperiodic, by placing identical unit cells at the points in space defined by vectors such as those defining ordinary crystal lattices. There is no language in which to search for common ground.

Disappointingly, the most promising candidate has now been used to suggest that the task of telling the true structure of icosahedral crystals is virtually impossible. This is one conclusion of the latest account by Per Bak, of Brookhaven National Laboratory, of his way of regarding icosahedral crystals as the projections on three-dimensional real space of crystal lattices of a more familiar kind constructed in six dimensions (*Phys. Rev. Lett.* 56, 861; 1986). Last year, Bak's account of how structures with only vestigial symmetry — such as the tiling of the two dimensional plane by two rhombi of different shape — may be related to more symmetrical structures in a higher dimension was one of the most stimulating features of the enquiry.

The argument has general interest. An icosahedron is a solid with 20 faces, each of which is an equilateral triangle. One way of visualizing the figure is to construct a pair of pentagonal pyramids and to bolt them together, one pentagonal base to the other, with a ring of ten equilateral triangles joined head to toe. There are

twelve vertices, all geometrically equivalent, at which the corners of five triangles come together. Icosahedral symmetry is characterized by a total of 120 symmetry operations. For making crystals, icosahedra are unsatisfactory building blocks. For one thing, it is impossible to fill three-dimensional space with them alone. For another, fivefold symmetry is literally incompatible with an underlying lattice structure of the Bravais type. And then there is no simple way of describing such a lattice, even if it could exist, in terms of integral multiples of some set of independent displacement vectors such as normally define the shape of a crystallographic unit cell.

The last difficulty, more than a mere annoyance, is most simply illustrated by a hexagonal lattice in two dimensions as in a sheet of carbon atoms from a graphite crystal. The simplest description is in terms of a pair of vectors of equal length making an angle of 60 degrees with each other. A little scribbling will show that this choice allows the construction of a two-dimensional crystal lattice in which the smallest unit cell is a rhombus whose sides are three times as long as the basic hexagonal sides; each contains two complete hexagons and bits and pieces enough to make a third.

So why not find a more natural description reflecting the inherent hexagonal symmetry? The natural choice of basis vectors would be the set of three equal vectors defined in direction by the three hexagonal sides that meet at every vertex. An arbitrary choice must be made between the two orientations of triple vertices that occur, but the more serious difficulty is that the three vectors are not independent. (If their directions are chosen to point outwards from a triple vertex, their sum will be zero.) In effect, Bak's way of dealing with this difficulty is to pretend that the chosen vectors are in reality independent which, because there are three of them, means that they span three-dimensional space. As in an ordinary crystal, lattice points are then assumed to occur at all points represented by integral multiples of the basis vectors, and the two-dimensional hexagonal pattern will be found by cutting a suitable two-dimensional plane through that three-dimensional lattice.

Last year, Bak successfully applied this technique to the case of icosahedral symmetry, where the natural basis vectors are

a set of six drawn from the centre of the figure to each of the vertices of a pentagonal pyramid chosen at random. (The other six vertices are reached by projecting these vectors backwards.) It does no harm if this set of basis vectors are visualized as orthogonal axes in six-dimensional space. The most general description of this crystal is by means of a function representing the likelihood that matter of a particular kind (manganese or aluminium atoms in the Schechtman case) will occur at some point in space, which must in turn be a periodic function reflecting the repetitiveness of lattice displacements. The underlying icosahedral symmetry is reflected in the way in which this density function can be represented by simple periodic functions such as sines and cosines; symmetrically equivalent Fourier coefficients are identical.

Getting from here to the real world is not so simple. The six-dimensional lattice has icosahedral symmetry, but real three-dimensional space is not related to that pattern in a simple or even unique way. The trick is to classify the different ways in which the symmetry of the six-dimensional symmetry will show up on the three-dimensional "hypersurface" which is the real world. The answer is that the positions of atoms in the real crystal will be determined by the intersection of a sequence of three-dimensional "surfaces" in six dimensions with the real three-dimensional world (itself a hypersurface).

Two conclusions are arresting. First, Bak shows that it is possible to recover the now-familiar Penrose tiling of the two-dimensional plane, and its three-dimensional analogue, as special cases of his six-dimensional construction. But he also shows that there are limitless other ways in which the appearance (in diffraction patterns) of icosahedral symmetry may be generated. The implications of that are stark. There is no "simple mathematical model" of the Schechtman alloy, and no alternative to the complete solution of six-dimensional crystal structures for those who wish to know where the atoms are in these quasi-crystals. But Pauling, who argues for the twinning of unit cells unable otherwise to fill space, will be glad to note that Bak offers the distortion of the "natural" unit cell as one way of looking at icosahedral symmetry. What nobody has yet considered seriously is the energetics of these odd forms.

John Maddox

Hot stars

Breaking through the wall

from James B. Kaler

STARS live such long lives that we cannot actually watch one age, even over many human lifetimes. Birth-to-death changes wrought by time must be reconstructed by observing many different stars in varying states of decay, and linking them together by age estimates and theory. The course of stellar evolution has been charted quite successfully: we can recognize young stars; we know what their end products will be; and we can see and understand the natures of many of the intermediate states. Yet there are gaps in the record. The discovery by Atherton, Reay and Pottasch reported in this issue (*Nature* **320**, 423; 1986) begins to fill one of the voids, by finally disclosing a member of a theoretically predicted class of extremely hot stars.

The subjects at hand are the stars in the intermediate mass range between about 0.8 and 6–10 times that of the Sun. Below the lower limit, the lifetimes are longer than the age of the Universe, so that none have yet died. Above the very insecure upper limit they explode. Those in the middle lead stable lives for a few billion years as they fuse hydrogen to helium in their cores, then go through a complex series of changes before expiring quietly as the incredibly dense remnants called white dwarfs. When the original hydrogen fuel is exhausted, the core contracts and heats while the outer envelope expands and cools to produce one of the commonly observed red giant stars. When the core temperature gets high enough, the helium created earlier can fuse to carbon, which for a while again stabilizes the star and allows the envelope to contract. On exhaustion of the helium, core contraction resumes, and the envelope expands once again into a giant state. Such a star can be so large (comparable to the inner Solar System) and the surface gravity so low that matter can be expelled in a fierce wind that removes most of the outer envelope, considerably diminishing the stellar mass. A final expulsion of matter reveals a nearly bare core, which is overlain with thin nuclear burning shells of helium and/or hydrogen, topped by a thin, non-reacting hydrogen skin. As this remnant envelope diminishes through winds and nuclear consumption from below, the surface temperature rises, and what is left of the star begins to contract. As it passes 30,000 K it begins to produce enough ultraviolet radiation to ionize the last expanding ejectum, and we now see a planetary nebula, a glowing cloud of gas surrounding a hot blue nucleus.

The stars are predicted to heat at a constant mass-dependent luminosity (Fig. 1)

until they reach a maximum temperature also dependent on mass, whence they cool and dim as they head for their final states among the white dwarfs that abound in

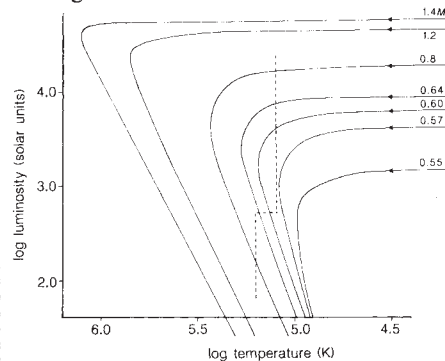


Fig. 1 Evolution of planetary nuclei in temperature and luminosity as a function of stellar mass (in solar units, $M_{\odot}$). The lowest four curves are from Schönberner (*Astr. Astrophys.* **79**, 108; 1979) and personal communication, 1983), the next two from Paczyński (*Acta Astr.* **21**, 417; 1971), and the highest is an extrapolation. Dashed lines give the location of Zanstra's wall, the high-temperature limit deduced from conventionally observed stellar magnitudes. (Adapted from R. A. Shaw, thesis, Univ. Illinois, 1985.)

space. This scenario is displayed with real nebulae in Fig. 2, where six planetaries are photographically placed at the same distance and arranged from left to right roughly in order of evolutionary state. The ones at the top are younger and the first three are on the horizontal tracks of Fig. 1. For the middle two we see bright stars in small nebulae, as expected. But as a star heats and produces relatively more ultraviolet photons, its nebula brightens considerably while the optical luminosity diminishes, and the star becomes lost in

the glowing cloud. Thus, we see nebulae with no apparent stars, as has been the case for the fourth object of the top line in Fig. 2, NGC2440, before the new work of Atherton and colleagues. Eventually, a nebula expands to the point where its now-cooling and much dimmer star is again revealed, as shown in the lower two photographs of Fig. 2.

Because most of the stellar radiation is in the far ultraviolet, temperatures from optical spectra are hard to find. Fortunately, we can use the fluorescing nebulae literally to count the number of ionizing photons, which when compared with optical luminosities, yield well-determined measures called Zanstra temperatures. When coupled with estimates of distances we can compute intrinsic luminosities and overlay the results on the theoretical evolutionary tracks to test the theory. With the best available optical stellar magnitudes (apparent luminosities), R.A. Shaw (thesis, Univ. Illinois, 1985) found a high temperature limit near 125,000 K, which he termed Zanstra's wall (outlined in Fig. 1). The stars of lowest mass fall nicely inside the wall, but those of higher mass become so hot that they cannot easily be found until they are quite dim: thus the gap in the observational record.

There have been many false or insecure results that have placed stars beyond the wall. Abell (*Astrophys. J.* **144**, 259; 1966), for example, gives temperatures to 250,000 K, but these are grossly inflated because of the unknowing inclusion of nitrogen spectrum lines into the nebular brightnesses used to count stellar photons. Similar results have been derived from old and unreliable magnitudes, many of which seem to be erroneous. Other, indirect, methods use nebular ionization or energy balance to infer high temperatures (for example, see Preite-Martinez A. & Pot-

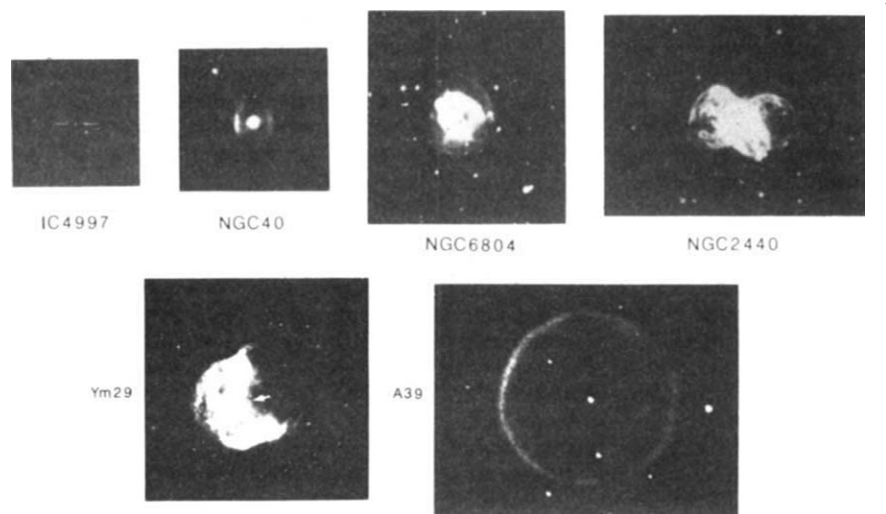


Fig. 2 Evolution of planetaries illustrated by six nebulae set at the same distance. From left to right the objects proceed in the direction of the arrows in Fig. 1. IC4997, Lick Observatory; NGC40, University of Illinois; NGC2440, Palomar Observatory; others, Kitt Peak National Observatory. (Panel adapted from Kaler, J. B. *Am. Sci.* in the press.)

tasch, *S. Astron. Astrophys.* **94**, 213; 1981), but the stars themselves are not directly examined. Satellite observations in the near ultraviolet indicate some extreme temperatures, but these result from observational error and difficulty in understanding the nature of the radiating stellar atmospheres. Atherton *et al.* (*Astrophys. J.* **232**, 786; 1979) located what may be a star of more than 200,000 K in a nebula similar to NGC2440, NGC7027, but the identification is only tentative.

We see then the significance of the new work by Atherton and colleagues — for the first time such a hot star has been unambiguously seen, and its temperature measured. Now the wall has been truly broken, pioneering the technique necessary to fill in one of the few remaining holes in the paths of stellar evolution. □

James B. Kaler is in the Department of Astronomy, University of Illinois at Urbana-Champaign, 1011 West Springfield Avenue, Urbana, Illinois 61801, USA.

Intracellular signalling

GTP and calcium release

from P. F. Baker

CALCIUM is an important intracellular signal, key features of the system being the maintenance of a low concentration of free Ca in the cytosol, usually in the region of 100 nM; the existence of pools of Ca, both intracellular and extracellular, at much higher concentrations; mechanisms for transferring Ca from these pools into the cytosol; and, finally, receptors in the cytosol that can respond to changes in free Ca in the 100–1,000 nM range. Although much is known about these receptors and how the various pools of Ca are established, surprisingly little is known about how Ca is permitted to move downhill from these pools into the cytosol. At the plasma membrane the problem seems to be solved by the existence of Ca channels that are gated either chemically or by voltage, and it has always seemed likely that something similar may exist to effect intracellular release; but only recently have possible mechanisms begun to emerge, one of which is presented by Gill *et al.* on page 461 of this issue¹.

The two major intracellular organelles that bind Ca are the mitochondria and the endoplasmic reticulum. Although their relative importance has long been debated, largely through the application of X-ray microanalysis to fast-frozen tissue, there now seems general agreement that in resting cells mitochondria contain rather little Ca but can sequester massive amounts should the cytosolic Ca begin to rise. In contrast, despite its relatively small Ca-binding capacity, the endoplasmic reticulum looks the stronger candidate for a high-affinity, physiologically relevant, intracellular Ca store. This comes as no surprise to muscle physiologists, who have long recognized that the myofilaments of skeletal and cardiac muscle are surrounded by a highly specialized derivative of the endoplasmic reticulum — the sarcoplasmic reticulum, which serves to regulate the local Ca concentration to which the Ca-sensitive contractile apparatus is exposed. Even in this tissue, however, which is apparently ideal

for experimental investigation, there is still considerable uncertainty about how Ca is released. Likely mechanisms for coupling electrical excitation to Ca release continue to be dominated by variations of the electro-mechanical, charged-plug model first put forward more than 10 years ago by Schneider and Chandler².

The rise to prominence of the endoplasmic reticulum has brought new ideas about Ca mobilization and there now seems to be more known about the mechanism of Ca release from this structure than from the sarcoplasmic reticulum. A major step forward was the discovery by Streb *et al.*³, rapidly confirmed in many laboratories, that the water-soluble molecule inositol trisphosphate (IP₃), a hydrolysis product of the membrane lipid phosphatidylinositol bisphosphate, can release Ca from the endoplasmic reticulum of many cells. In one step, this provided a link between membrane receptors and release of Ca from a major intracellular store. All subsequent work points to the existence in the endoplasmic reticulum of some sort of IP₃-gated calcium channel. The obvious and very interesting possibility that IP₃ may also be involved in releasing Ca from the sarcoplasmic reticulum of skeletal muscle (see the recent discussion in these columns⁴) received some early and dramatic support but has so far failed to gain general acceptance. A subtle variant however, such as an IP₃-type molecule as part of the Schneider-Chandler plug acting to release Ca via an IP₃-type receptor in the sarcoplasmic reticulum is still an open possibility. In addition, by the isolation of nucleotide-gated channels, Smith *et al.*⁵ have given new impetus to the search for physiologically relevant, chemically gated Ca channels in the sarcoplasmic reticulum.

Now, Gill *et al.*¹ interpret some very interesting new data in terms of another mechanism. Working with a detergent-permeabilized neuroblastoma cell line, they report that approximately half of the Ca accumulated by a store, presumed to

be the endoplasmic reticulum, can be released by exposure to micromolar concentrations of GTP. This very clear effect is highly specific for GTP and cannot be mimicked by non-hydrolysable analogues suggesting that GTP hydrolysis might be an essential part of the Ca-release process. GTP-dependent release is temperature-dependent and can be inhibited competitively by GDP. The authors make a plausible case for the involvement of a GTP cycle in the control of calcium permeability in the endoplasmic reticulum of neuroblastoma cells. They fail to explain, however, why their system is not permanently activated by the levels of GTP always present inside these cells.

What is the relation between GTP-induced and IP₃-induced Ca release? The authors claim they are two separate systems. Thus, although IP₃ is active in their cells, its effects are not inhibited by GDP and exhibit a different temperature dependence from GTP-induced release. Although suggestive, these arguments are not compelling and, as the authors admit, it is possible that the IP₃- and GTP-dependent systems for releasing Ca may prove to be closely related. The finding of Dawson⁶ that IP₃-induced release is strongly stimulated by GTP might be highly significant in this respect. GTP-binding proteins are fast coming to occupy a central position in coupling membrane events and their classic role in coupling occupied receptors to cyclase activation now finds parallels in the activation of phospholipase C and even the opening of channels^{7,8}, so there is ample precedent for a similar role in effecting Ca release from the endoplasmic reticulum. According to this view, the preparation of Gill *et al.* may contain an endogenous ligand that can, in the presence of GTP, open Ca channels in the endoplasmic reticulum. The requirement for both GTP and an unknown ligand would both circumvent the tricky question of why physiological levels of GTP do not keep the channels open permanently and focus attention on the nature of the hypothetical intracellular ligand, the binding of which is coupled by GTP to channel opening. If such a ligand exists, it could still be IP₃ or a close relative. The onus seems to be on Gill and his colleagues to prove it does not exist. □

- Gill, D. L., Ueda, T., Chueh, S.-H. & Noel, M. W. *Nature* **320**, 461 (1986).
- Schneider, M. F. & Chandler, W. K. *Nature* **242**, 244 (1973).
- Streb, H., Irvine, R. F., Berridge, M. J. & Schulz, I. *Nature* **306**, 67 (1983).
- Somlyo, A. P. *Nature News and Views* **316**, 298 (1985).
- Smith, J. S., Coronado, K. & Meissner, G. *Nature* **316**, 446 (1985).
- Dawson, A. P. *FEBS Lett.* **185**, 147 (1985).
- Pfaffinger, P. J., Martin, J. M., Hunter, D. D., Nathanson, N. M. & Hille, B. *Nature* **317**, 536 (1985).
- Holz, G. G., Rane, S. G. & Dunlap, K. *Nature* **319**, 670 (1986).

P. F. Baker is Halliburton Professor of Physiology, King's College London, Strand, London WC2R 2LS, UK.

Earth sciences

Growing stromatolites

from Peter J. Smith

CONTRARY to popular belief, terrestrial life did not begin at the start of the Cambrian, about 600 million years (Myr) ago, or even during the few hundred million years immediately preceding it. The Precambrian–Cambrian boundary simply represents the time at which abundant organisms developed hard parts capable of leaving fossil remains. Evidence for what meagre life there was during most of the Precambrian exists not as skeletons and shells but largely as stromatolites, sedimentary structures produced by the activity of blue-green algae. A new discovery by Gomes (*Trans. geol. Soc. S. Afr.* **88**, 1; 1985) reminds us that stromatolites differ from many organisms in the fossil record in that they are still being produced in limited numbers today, giving some insight into the Precambrian conditions under which they developed.

It is highly probable that the most primitive terrestrial organisms originated as long ago as the Hadean, the interval between the creation of the Earth and the formation of the oldest known rocks (about 4,600–3,800 Myr ago): there is certainly evidence for very ancient life in the form of microscopic filaments and cells of bacteria and blue-green algae in South African chert deposits at least 3,200 Myr old. Stromatolites are more important than these insofar as, having hard (albeit inorganic) parts, they have greater survival potential and are thus more common. Walter (*Stromatolites* Elsevier, Amsterdam, 1976) defined them formally as “organo-sedimentary structures produced by sediment trapping, binding and/or precipitation as a result of the growth and metabolic activity of micro-organisms”. In other words, they are not fossils at all in the conventional sense in that they are not primary organic remains, although without organisms they would not have been able to form. They are traces of past life much as burrows and tracks are, although more substantial than either.

The organisms involved in the generation of stromatolites are usually blue-green algae which, being filamentous, attract and bind surrounding particles of carbonate into an algal mat. The algae then extend their filaments throughout the newly accreted carbonate layer, attract more particles, and thus expand into thinly laminated structures which can be of considerable overall size. (The largest known forms are mounds hundreds of metres across and tens of metres high.) The algal layers themselves are seldom preserved, of course; but the carbon-

ate accretions often survive in various distinctive forms, such as tabular, domed, branch-like and columnar.

Stromatolites were produced during the Archaean (earlier than 2,500 Myr ago), reached their peak of abundance and diversity during the Proterozoic (2,500–600 Myr), and thereafter declined, although they are sometimes encountered in the Phanerozoic. Indeed, they are still being generated today in a few suitable marine and freshwater environments; modern examples are not so common that new discoveries are of merely routine interest. Despite a resurgence of interest in stromatolites during the past 20 years, the influences on their formation are still unclear, a point worth remembering in view of the many problems to which stromatolite data have been offered as solutions. Claims for the use of such data have been made in almost every branch of the earth sciences and even in astronomy, but as Hofmann (*Earth-Sci. Rev.* **9**, 339; 1973) has pointed out, not all the conclusions rest on sufficiently strong evidence to warrant their acceptance.

The new discovery by Gomes of two types of stromatolite currently growing in South Africa is thus of considerable interest, not least because the study reveals in detail the conditions under which growth is taking place. The site in question is a collapse sinkhole, Wondergat, in dolomite sediments in the western Transvaal. The hole has a cross-sectional area of about 2,000 m² and is about 50 m deep at the centre, although two long caves running off from the bottom edge extend the overall depth to about 70 m. More crucially, running off from the side wall at a depth of about 20 m is a smaller cave that has been dissolved into the boundary between the dolomites of the upper 20 m and the dolomitic limestone (with chert bands) underlying them.

The sinkhole contains fresh, clear water at a depth of about 10 m and a temperature of $21 \pm 4^\circ\text{C}$. The pH value of the upper 15 m of water (the depths at which stromatolites grow) is between 7.38 and 7.76, the Ca²⁺ concentrations vary from 53 to 68 p.p.m (parts per million) and Mg²⁺ concentrations range from 2.7 to 3.4 p.p.m. Water movement is slight, and natural visibility is high at distances of 10–15 km.

It is not possible to tell which of these chemical characteristics are necessary for stromatolite growth. For example, if all other circumstance were to remain unchanged, would stromatolites grow if the pH of the water were, say, 7.2 or 7.9? Or to put the question in a slightly different

way, is the greatest depth of stromatolite growth governed solely by the ability of sunlight to penetrate and hence to allow the photosynthesis necessary for the production of the algae, or is it also defined or limited by one or more of the chemical properties of the water?

As for the stromatolites themselves, tabular ‘crinkled’ forms grow abundantly on the dolomite side walls of the sinkhole in the upper 15 m of water. They are 15–20 mm thick; light-brown to greenish in colour; soft and spongy; and, apart from the living algae, consist mainly of CaCO₃. Columnar stromatolites grow on the roof, walls and floor of the first 20 m of the smaller cave and in crevices in the wall of the main sinkhole. These vary in thickness from a few millimetres to a few centimetres; are the same colour as the tabular forms; and similarly consist mainly of CaCO₃, although they are hard, in contrast to the tabular stromatolites. The form of stromatolite appears to be governed by the range of genera involved, which is in turn influenced by the degree of sunlight available. Tabular forms grow where the light is brightest and columnar forms where it is relatively dim.

One curiosity of the Wondergat stromatolites is that they seem to need permanent submersion for survival and growth. If the water level falls, the exposed stromatolites rapidly desiccate. In this respect they differ from ‘typical’ stromatolites, which are thought to form with episodic immersions to allow the alternating layers of algae-rich and carbonate-rich material to be built up. There seems little doubt that most known stromatolites were generated in shallow-water zones where tides are the clearly recognizable agents for the periodic transport of sedimentary particles into the algal mats.

There have been previous claims for the discovery of deep-water stromatolites. Achauer and Johnson (*J. sedim. Petrol.* **39**, 1466; 1969), for example, concluded that stromatolites associated with a late Cretaceous reef in Texas must have been generated in deep water. In such a case, the influx of carbonate particles would not necessarily be sedimentary in nature. It is well established that photosynthesis by algal colonies can, by removing CO₂ from the water, increase the pH and accentuate the precipitation of CaCO₃ which then intermingles with the algae.

Gomes himself makes little comment on the wider implications of his data, which is perhaps wise. Modern stromatolites are quite rare, and freshwater occurrences make up only a very small fraction of the total, calling into question whether the Wondergat find is in fact typical of stromatolites. □

Peter J. Smith is Reader in Earth Sciences at the Open University, Milton Keynes MK7 6AA, UK.

Molecular biology

Transposon tricks revealed

from Andy Flavell

THE mobility of DNA sequences called transposons that can move around within the chromosome of a cell and its progeny has many genetic consequences. One particularly interesting transposon is the P element of the fruitfly *Drosophila melanogaster*, which is transposed (inserted at a new site on the chromosome) at a very high frequency. P elements are especially fascinating because they only transpose in a particular genetic environment; that is to say they are mobilized only when the transposon is placed in an egg that has no P elements already present. Mobilization occurs only in the germ line of the developing embryo (somatic tissue is unaffected). Results reported in two recent papers^{1,2} expose the way in which the P element achieves this.

Like all autonomously mobile elements, the P element encodes a transposase enzyme which mediates its transposition. Studies by Rubin's group have shown that P elements contain four open translational reading frames, each of which is necessary for transposase function³. It therefore seemed very likely that the entire protein-coding portion of the P element specified a single transposase enzyme. To determine whether the restriction of P transposition to germ tissues is caused by a transcriptional block, Laski, Rio and Rubin¹ have now replaced the P-element promoter with a heat shock protein (hsp 70) promoter known to be active in many tissues. Following the introduction of this construct into the *Drosophila* germ line, massive amounts of P-element RNA are synthesized in somatic tissues of flies descended from transformed parents, but no somatic transposition is observed. Furthermore, a study of natural P-element transposition revealed that the P promoter functions perfectly well in somatic tissues. The authors therefore reasoned that P transposition must be repressed at a post-transcriptional stage.

To resolve this problem the authors embarked on a detailed study of P transcription in somatic tissues (it is technically almost impossible to obtain sufficient quantities of germ cells from *Drosophila* embryos for transcript analysis). They discovered that the first three long open reading frames of the element¹ were spliced to each other as predicted but that no detectable splice existed between the third and fourth open reading frames (see figure). Therefore, a polypeptide synthesized from the RNA would be terminated by a stop codon before reaching the final reading frame, generating a protein of relative molecular mass 66,000 (66K). But

work from the same laboratory¹ had already shown this last reading frame to be essential. Laski, Rio and Rubin¹ therefore hypothesize that the block to P transposition lies in RNA splicing — in somatic tissue only a non-functional downstream truncated protein is synthesized; perhaps in germ tissue the last splice is correctly made to generate active transposase.

To test this hypothesis the authors removed the offending intron at the DNA level by oligonucleotide site-directed mutagenesis. The mutated P element (hsp-PΔ2-3) was then introduced into the *Drosophila* germ line and shown to be functional in both germ and somatic tissues, causing somatic mosaics.



The P element has four long open reading frames (ORFs). The somatic splicing pattern of the P-element RNA (Ps) generates a 66K polypeptide. The putative germ-line splicing of the P element (Pg) generates an 87K protein. The eight base duplications of host DNA (►) flanking the element are marked.

The successful release of P transposase from the shackles imposed on its expression gave Rio, Laski and Rubin² the opportunity to study this enzyme. Introduction of hsp-PΔ2-3 into cultured *Drosophila* cells results in the inducible synthesis of large amounts of an 87K protein. The nature of this protein was confirmed immunologically using antisera raised against P open reading frame polypeptides, and enzymatically by an elegant transposition assay using excision of a P element from the *Escherichia coli* β-galactosidase gene on a rescuable shuttle vector. The authors, with commendable modesty, 'tentatively' refer to this polypeptide as the transposase. In line with their earlier interpretation, the element containing a normal complement of introns can only direct the synthesis of a 66K protein that lacks transposase activity.

The possibilities for further study on P transposase are now readily evident. Bulk preparation of active transposase, either from *Drosophila* or bacterial cells, will allow a thorough biochemical study of the enzyme and the *in vitro* dissection of the transposition mechanism. To whet our appetites, Rio *et al.*² used the excision products of the rescued shuttle vectors to demonstrate the effect of alterations to the flanking eight-base duplications of the P element on the fidelity of excision. They

discovered that this perturbation yielded only imprecise excisions as opposed to a 25 per cent level of precise excisions of the normal element. The reason for such high imprecise excision of even the normal P element is unknown, but similar results are found in other transposons in plants⁵.

Alternative splicing pathways are well known nowadays, but an all-or-none splicing event is much rarer. What is the function of the 66K protein? Rio *et al.* point out that a tentative DNA-binding domain of this protein is present in both (66K and 87K) proteins and suggest that the 66K protein may inhibit P element transposition by competing with transposase for the termini of the element or by direct protein-protein interaction. The fact that P elements are apparently expressed in somatic tissues as a non-functional polypeptide seems wasteful. Could it have a function in the germ cells? Co-injection of a mutated P element that could produce only the 66K protein, together with a helper-P element, produces germ-line transformants¹, suggesting that the 66K protein does not completely inhibit transposase function in germ cells. Happily, models for the functions of these proteins can be tested now that they can each be produced in large amounts.

There are several lines of circumstantial evidence to suggest that P elements are not native to *D. melanogaster* but have invaded the organism in the past few hundred years. Many *D. melanogaster* fly stocks lack functional P elements and they are missing from closely related sibling species. But they are present in the distantly related *D. paulistorum*⁶, suggesting a horizontal transmission event. The rampant transposition of this element when introduced into *D. melanogaster* flies lacking the P element suggests that P elements are less fully adapted to their host than the more placid retrotransposons such as *copia* and *gypsy*. Perhaps P elements have not yet developed a germ-line specific promoter but have been able to take advantage of a pre-existing splicing control mechanism to protect the somatic tissues of their host from their ravages.

One final exciting possibility mentioned by the authors is that hsp-PΔ2-3 may transpose in cells from organisms other than *Drosophila*, perhaps even in the germ line and soma of vertebrates. If this turns out to be true then all the advantages given to *Drosophila* genetics by the P element will be available to vertebrate molecular biologists. □

1. Laski, F.A., Rio, D.C. & Rubin, G.M. *Cell* **44**, 7 (1986).
2. Rio, D.C., Laski, F.A. & Rubin, G.M. *Cell* **44**, 21 (1986).
3. Karess, R.E. & Rubin, G.M. *Cell* **38**, 135 (1984).
4. O'Hare, K. & Rubin, G.M. *Cell* **34**, 25 (1983).
5. Saedler, H. & Nevers, P. *EMBO J.* **4**, 585 (1985).
6. Daniels, S.B. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **81**, 6794 (1984).

Andy Flavell is a lecturer in the Department of Biochemistry, The University of Dundee, Dundee DD1 4HN, UK.

Immunology

Contrasuppressor cells and oral tolerance

from R.B. Taylor

AMONG the subtle regulatory tasks required of the immune system is adjustment of the modality and magnitude of an immune response to suit the anatomical locality in which it is to take place. A good example of this ability is oral tolerance — immune responsiveness to antigens at mucosal surfaces with a much reduced response to systemic challenge with the same antigens¹. The value of this mechanism is probably to prevent systemic allergic responses to food antigens without compromising local defence of the mucosae. The demands of such subtle functions should have prepared us for the further complexities in immunoregulatory mechanisms that continue to be revealed. One of these, which has now come to stay, is the contrasuppressor circuit². I. Suzuki and co-workers, on page 451 of this issue, now report data implicating contrasuppression in the maintenance of oral tolerance³.

As so far defined in mice, this circuit comprises T lymphocytes called contrasuppressor cells whose end function is to neutralize the inhibitory action of T-suppressor cells on the T-helper cell population. One of the cells necessary for contrasuppression has a distinctive surface phenotype and the property of adherence to *Vicia villosa* lectin. These cells have been found in various lymphoid organs including Peyer's patches and seem to play a part in selection of the modality (contact sensitivity or antibody production) of the response to the trinitrophenyl hapten^{2,4}. In man there is evidence of contrasuppression in cultures of peripheral blood lymphocytes responding to an antigen from the oral bacterium *Streptococcus mutans*. The cells responsible for this activity were shown to bind antigen and *V. villosa* lectin and carry the surface markers T8, T3 and Ia (see ref. 5 for review).

The work now reported by Suzuki *et al.*³ builds on the earlier finding in the same laboratory that oral tolerance could be induced in C3H/HeN mice but not in the congenic strain C3H/HeJ which differs at a locus determining the ability to respond to bacterial lipopolysaccharide. In the present work, mice of both strains are subjected to prolonged oral immunization with sheep red blood cells (SRBC). Oral tolerance develops in the C3H/HeN mice but can be broken by transfer of T-cell fractions enriched for contrasuppressor cells taken from the spleens of the C3H/HeJ mice — to result in the development of anti-SRBC antibody-forming cells of all

the major isotypes in the recipient spleen. Very small numbers of contrasuppressor cells (5×10^4) are effective. Oral tolerance to SRBC is also shown by C3H/HeN spleen cells *in vitro*, and is similarly abrogated by the inclusion of contrasuppressor cells in the cultures. On the basis of various methods to enrich or deplete the active cells, it was concluded the contrasuppressor cells resemble those originally described. The fact that oral tolerance to SRBC is lost in the presence of contrasuppressor cells indicates clearly that T-helper cells are present but controlled by suppressor cells. If this suppression is neutralized by the contrasuppressor cells then T-helper cells can become active.

The phenomenon of local immune responses in the gut coexisting with oral tolerance has been described as "an island of responsiveness in a sea of suppression".

Astronomy

A hidden quasar revealed

from C. Martin Gaskell

IT has long been realized that the quasar phenomenon encompasses many orders of magnitude in luminosity, ranging from the brightest quasars, which far outshine the galaxies they are in, down to the mildly active nuclei (often called mini-quasars) of relatively normal galaxies like our own, that have luminosities comparable with the brightest stars. Despite the enormous range in energy output (a factor of at least 10^{10}) there are many features that all these active nuclei possess in common; the most important is a spectrum with (roughly) constant energy per decade of frequency in the entire range from millimetre wavelength radio waves to X-ray (and probably γ -ray) energies. Does this mean that all such 'active' galactic nuclei are manifestations of the same basic quasar mechanism? The answer in recent years has been an increasingly firm yes. And R.R.J. Antonucci and J.S. Miller¹ have now made observations greatly strengthening our belief in the unity of quasar activity.

The quasar family is divided into several genera and species on the basis of the strength and nature of the radio emission and the optical emission line spectrum. One of the more important species is the Seyfert 2 galaxy. A Seyfert galaxy has emission lines whose Doppler broadening

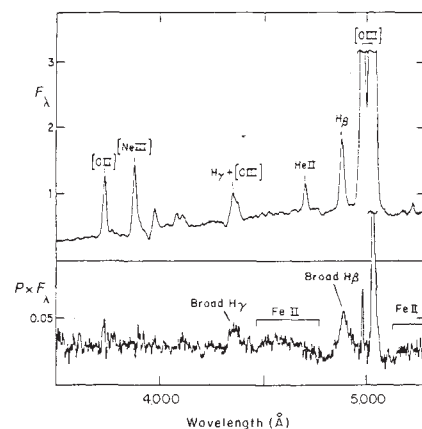
The challenge now is to find out what elements of the system are sufficiently localized to determine the geography of this island. Restricted migration of contrasuppressor cells from the gut seems unlikely as these cells are found in the spleens of the C3H/HeJ mice after oral immunization. Instead one may need to look at the populations of cells which, once primed, return to the mucosae to function in local immunity. It may be that there is a defect in the return of T-suppressor cells relative to the other types. The growing interest in mucosal immunization is to be welcomed because the mucosal route is after all the commonest, whereas intraperitoneal immunization rarely occurs outside the laboratory — except perhaps in the bull-rings of Seville⁶. □

1. Challacombe, S.J. & Tomasi, T.B. *J. exp. Med.* **152**, 1459 (1980).
2. Green, D.R., Flood, P.M. & Gershon, R.K. *A. Rev. Immun.* **1**, 439 (1983).
3. Suzuki, I., Kiyono, H., Kitamura, K., Green, D.R. & McGhee, J.R. *Nature* **320**, 451 (1986).
4. Ptak, W., Bereta, M., Marcinkiewicz, J., Gershon, R.K. & Green, D.R. *J. Immun.* **133**, 623 (1984).
5. Lehner, T. *Immun. Today* (in the press).
6. Coutinho, A., Formi, L., Holmberg, D., Ivars, F. & Vaz, N. *Immun. Rev.* **79**, 151 (1984).

R.B. Taylor is in the Department of Pathology, University of Bristol, Bristol BS8 1TD, UK.

exceeds the velocities of normal interstellar gas and stars in the rest of the galaxy. Khachikian and Weedman² sub-divide Seyfert galaxies into two classes: Seyfert 1, where ionized gas moves very rapidly (velocities of a few per cent of the speed of light); and Seyfert 2, where the ionized gas moves only slightly faster than the stars in a typical galactic nucleus (an order of magnitude slower than in Seyfert 1 galaxies). Seyfert 1 galaxies are clearly closely related to the bright quasars. The dense rapidly moving gas producing the strong broad emission lines (the so-called broad-line region) is an intimate part of the quasar phenomenon. It is found at radii as small as a few light days from the energy source in some Seyfert 1 galaxies³ (only a few times bigger than the Solar System) and is seen in all bright quasars. Why is it not seen in Seyfert 2 galaxies? There are two probable reasons: either the dense gas is somehow hidden; or it is absent, in which case Seyfert 2 galaxies could be fundamentally different from bright quasars.

In the past few years it has been realized that Seyfert 1 galaxies can masquerade as Seyfert 2 galaxies either by temporarily turning off their photoionizing continuum (see, for example, ref. 4) or, in some cases by having a lot of dust obscuring their innermost regions^{5,6} (dust is certainly pre-



Spectra of NGC1068 showing total flux (top) and polarized flux (bottom). The hidden broad lines are only revealed in the polarized light. (Adapted from ref. 1.)

sent in most Seyfert 1 galaxies). But the difference between Seyfert 1 and Seyfert 2 galaxies is more fundamental than the latter simply being turned-off or hidden Seyfert 1 galaxies as the latter have more powerful and extended radio emission⁷.

New optical spectropolarimetric observations of NGC1068 by Antonucci and Miller¹ have gone a long way towards answering the question of what is a Seyfert 2? NGC1068 is the brightest and nearest Seyfert 2 galaxy showing little variability and with no obvious heavy dust obscuration in the nuclei (see figure). It is thus a 'pure' Seyfert 2. Antonucci and Miller have obtained high-quality spectropolarimetric data with the Lick Observatory 120-inch telescope. Looking at only the polarized light they made a remarkable discovery — the light shows the unmistakable spectrum of a Seyfert 1, or quasar, with its high-density rapidly moving gas. This prototypical Seyfert 2 therefore contains a hidden quasar, which is revealed

because, although the light from the innermost region cannot reach us directly, it can scatter off free electrons around the active region. This scattered light, although feeble, is highly polarized.

The photoionizing continuum light that can be observed must be reaching us in the same indirect way, as it shares the same polarization as the hidden broad lines. Antonucci and Miller conclude that the central quasar is probably hidden by a large, thick, doughnut-shaped disk whose hole corresponds to the axis of ejection of the radio-emitting plasma (it has long been known that the optical polarization angle of quasars is related to the orientation of the radio structure^{8,9}). Their observations are the best evidence so far that such disks exist.

The Lick Observatory discovery is therefore important for at least two reasons: it tells us a lot about the architecture of Seyfert 2 nuclei; and it also strengthens the case for the basic unity of all species of the quasar family and emphasizes the importance of broad-line regions. What is happening at the centre of NGC1068, as revealed in the ghostly polarized optical spectrum, looks exactly the same as what is going on in any bright quasar. □

1. Antonucci, R.R.J. & Miller, J.S. *Astrophys. J.* **297**, 621 (1985).
2. Khachikian, E. Ye. & Weedman, D.W. *Astrofizika* **7**, 389 (1971).
3. Gaskell, C.M. & Sparke, L.S. *Astrophys. J.* (in the press).
4. Lyutyi, V.M. Oknyanskii, V.L. & Chuvayev, K.K. *Soviet Astron. Lett.* **10**, 335 (1984).
5. Lawrence, A. & Elvis, M. *Astrophys. J.* **256**, 75 (1982).
6. DeZotti, G. & Gaskell, C.M. *Astron. Astrophys.* **147**, 1 (1985).
7. Ulvestad, J.S. & Wilson, A.S. *Astrophys. J.* **285**, 439 (1984).
8. Stockman, H.S., Angel, J.R.P. & Miley, G.K. *Astrophys. J.* **227**, L55 (1979).
9. Antonucci, R.R.J. *Nature* **303**, 158 (1983).

C. Martin Gaskell is in the Department of Astronomy, Ohio State University, Columbus, Ohio 43210, USA

Plate tectonics

Deformation of continents

from Philip England

ALTHOUGH many geologists now claim at least a broad understanding of the rules governing the global plate-tectonic system, few would be so bold about the dynamics of the large mountain belts that mark the convergence of continental portions of the plates. In contrast to the oceans — where the acquisition of major new sets of data led rapidly to the theory of plate tectonics — the problem in interpreting the geological record on land appears to be an excess of observations, and a lack of a unifying framework in which to view them. In part, this problem arises because the success of plate-tectonic theory in the oceans tempts one to apply the same rules to the continents, but although plate motion is responsible for continental

collision, the resulting deformation does not appear to be explicable by the relative motion of a few rigid plates. Characterizing the deformation in a more useful fashion is difficult because of the number of different perspectives with which mountain belts are viewed.

A recent meeting* brought together petrologists, structural geologists and geophysicists to discuss theory and observation of orogenic (mountain-forming) belts. The most contentious issue was the configuration of deformation and metamorphism, with most advocating the view that continental tectonics is the relative movement of many rigid blocks, but a few

wishing to view it as a quasi-continuous process. The observations receiving most emphasis were those that bore on this question, those relating to the physical conditions of metamorphism and those suggesting that extensional tectonics may be important in the preservation of metamorphic assemblages generated in compressional orogenic belts.

The inference, from the compositions of minerals formed under disequilibrium conditions in transient thermal regimes, of the heat sources for metamorphism is a long-standing problem in petrology, and one that seems to become less tractable as more (and more sophisticated) data become available. Much of the complexity in the metamorphic record results from syn- to post-tectonic movements (for example, faults, shear zones and doming) on a scale that is much smaller than that of the crust as a whole, and disproportionate attention is paid to such metamorphically complicated regions (B. Hart and T. Dempster, Edinburgh University; A. Thompson and J. Ridley, Federal Institute of Technology, Zürich). These zones of disruption often bound larger regions that record relatively simple metamorphic histories which, although they may be less challenging to the thoroughbred petrologist, are more likely to elucidate the thermal conditions of metamorphism.

The continental lithosphere deforms over regions of hundreds of thousands of kilometres in width, but seismic and geodetic observations show that, within the brittle upper crust, this deformation is broken up on a scale of a few to a few tens of kilometres (J. Jackson, Cambridge University; R. Walcott, Victoria University, Wellington) and metamorphic observations show a similar scale of fragmentation (B. Harte and T. Dempster; A. Thompson and J. Ridley; B.W.D. Yardley *et al.*, Leeds University). Some participants, including M. Parmentier (Brown University) and myself, held that this scale is sufficiently small, compared with the total size of deforming belts, that some understanding of the dynamics could be obtained by treating the continents as continuous media, but much of the discussion (focused by E. Artyushkov, USSR Academy of Sciences, Moscow) found this an oversimplification.

The tectonic history of New Zealand seems to exemplify all the complexity that is claimed for the deforming continents: since the Eocene, relative motion between the Pacific and Australian plates near New Zealand has been accommodated by a combination of compressional, extensional and transcurrent motion over a belt several hundreds of kilometres wide. Yet geodetic observations of the strain during this century indicate that it is continuous when viewed on a scale of about 50 kilometres, and this strain, if it had operated over the past 15 million

*Tectonic Settings of Metamorphism Royal Society Discussion Meeting, 29–30 January 1986.

years, would be consistent with the palaeomagnetically determined rotations of post-middle-Miocene rocks in the region (R. Walcott, Victoria University).

A superficial examination of the thermal conditions beneath the North Island of New Zealand suggests that it is a classic paired metamorphic belt, but in a tectonic setting not usually attributed to these belts: high temperature–low pressure (high T –low P) metamorphism is occurring beneath the central volcanic region, which is extending and subsiding, while low T –high P metamorphism is occurring in the Hikurangi Margin to the east, which is uplifting and extending. Thus, a future episode of compression is required before erosion can expose the high T –low P metamorphism, whereas the exhumation of low T –high P rocks, which is usually thought to require the cessation of subduction, requires no more than the

continuation of the present underplating and extension of the Hikurangi Margin.

A similar process is believed to have operated in the preservation of high-pressure rocks in the Franciscan Formation, Betic Cordillera and Western Alps (J. Platt, Oxford University); large normal faults parallel to the mountain range are also observed in the High Himalaya (J.-P. Burg, Melbourne University). But nobody was prepared to propose such an extensional mechanism for the preservation of a 50-square kilometre area of coesite-bearing rocks that appear to have been metamorphosed at about 30 kilobars in the early stages of the Alpine orogeny (C. Chopin, Ecole Normale Supérieure, Paris). □

Philip England is in the Department of Geological Sciences at Harvard University, Hoffmann Laboratory, 20 Oxford Street, Cambridge, Massachusetts 02138, USA.

Palaeontology

How did eurypterids swim?

from D.E.G. Briggs

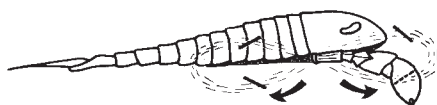
EURYPTERIDS, a group of extinct Palaeozoic arthropods, existed for more than 200 million years from the early Ordovician to the Permian, and were the largest arthropods ever to have evolved—at least half of the eurypterid families included species containing individuals of more than 80 cm long. They became extinct in the most severe mass extinction in the late Permian. A recent report suggests that these large swimming arthropods may have been capable of hovering (Plotnick, *R. Trans. R. Soc. Edinb.* **76**, 325; 1985).

Eurypterids had a long streamlined body divided into an anterior prosoma (cephalothorax) and posterior opisthosoma (abdomen). Trace fossil evidence and functional analysis show that eurypterids used the posterior three pairs of prosomal appendages to walk in a hexapodous gait. The last of these, the sixth prosomal appendage, was modified to some extent in all eurypterids, the distal podomeres being commonly flattened and expanded to form a paddle.

Early interpretations of swimming in eurypterids were based largely on comparisons with the horse-shoe crab (*Limulus*), a member of the only major living group of aquatic chelicerates, and envisaged eurypterids as swimming ventral side uppermost using mainly the opisthosomal limbs (equivalent to the gills of *Limulus*). More detailed studies of the few well-preserved examples (Waterston, C.D. *Fossils and Strata* **4**, 241; 1975) showed that eurypterid gills were more similar to the lung sacs of scorpions, consisting of an area or tract on the ventral body wall, rather than appendages modified as book-gills. These

tracts were protected by stiff cuticle and could not have been used for propulsion.

Recent research demonstrates that the paddle provided the primary means of swimming. Paul Selden redescribed the Silurian eurypterid *Baltoeurypterus tetragonophthalmus* (*Trans. R. Soc. Edinb.* **72**, 9; 1981) based on the spectacular fossil specimens from the Silurian of Gotland



Hovering in *Baltoeurypterus*. The paddle is moved in a shallow horizontal figure-of-eight, the angle of attack (dashed on the paddle, solid elsewhere) altering to maintain lift. Other prosomal limbs are omitted. This specimen is about 20 cm long.

and Estonia, in the Baltic. The eurypterid cuticle can be isolated from limestone by dissolution in acid and provides information comparable to the exoskeleton of a living arthropod. Most importantly, the mechanics of the joints between the limb podomeres can be reconstructed.

Appendicular swimming may be either predominantly drag-based (the appendages rowing, as oars) or lift-based (the appendages 'flying', as hydrofoils). Selden's analysis of the mechanics of the paddle in *Baltoeurypterus* indicates that the large flat seventh and eighth podomeres could have been alternately expanded to create maximum drag in a power stroke, and then rotated about the paddle and folded to present much less resistance in a recovery stroke. He suggested that the paddles functioned as oars, moving in phase to reduce the likelihood of yawing;

pitching and rolling would have been prevented by the flattened body, tergal projections and outstretched limbs.

But *Baltoeurypterus* was about 20 cm long, a size that exceeds the limit at which drag-based swimming becomes inefficient (at Reynolds numbers of about 10^3). The limited degree of lift produced by rowing would also be an important consideration if eurypterids were negatively buoyant, as seems probable. Using Selden's detailed analysis of the morphology of *Baltoeurypterus*, Plotnick's new work extends the model of swimming in eurypterids to incorporate a greater element of lift. Plotnick's conclusions are based largely on a study of swimming in portunid crabs, which have a paddle (the fifth pereopod) strikingly similar to that in eurypterids. Portunids are very manoeuvrable, using a lift-based swimming mechanism that even enables them to hover.

Eurypterids were unable to swim by 'subaqueous flying' (in the manner of penguins) because the nature of the joints prevented up-and-down movement of the paddle at right angles to the direction of progress. But they could move the paddle in a flattened figure-of-eight which, while concentrating the thrust in the backstroke as in rowing, produced lift and a modest degree of thrust in the forestroke. The paddle tapered to a point distally, like a hydrofoil. (It should be noted, however, that the morphology of the paddle is likely to be a compromise between the different requirements of swimming and walking.) Lift-based swimming is more efficient for a large animal than rowing, as a propulsive thrust is produced on both the forestroke and the backstroke and a constant lift is created to counteract the negative buoyancy of the animal. In the eurypterids, the dorsal position of the articulation between podomeres 4 and 5 exaggerated the downward component of the paddle's movement, thus favouring lift.

Not all eurypterids conformed to the morphology of *Baltoeurypterus*. The remarkable late Silurian pterygotids, for example, were more elongate with very slender limbs (including the paddles) and may have been more than 2 metres long. This large size indicates that a lift-based mode of swimming was likely (Selden, P.A. *Spec. Pap. Palaeont.* **32**, 39; 1984).

Only a few eurypterids have been subjected to the kind of detailed analyses pioneered by Selden and Plotnick. Although material of the quality of *B. tetragonophthalmus* from the Baltic is rare, research on the biomechanics and hydrodynamics of other well-preserved eurypterids will yield further insights into the functional strategies adopted by these remarkable arthropods. □

D.E.G. Briggs is in the Department of Geology, University of Bristol, Wills Memorial Building, Queen's Road, Bristol BS8 1RJ, UK.

Outlook brighter on weather forecasts

SIR—Our recent paper "Fractal characterization of inhomogeneous geophysics measuring networks" addressed the fundamental geophysical problem of sparse measuring networks, and pointed to new ways of overcoming longstanding difficulties¹. Although pleased to have stirred interest in the weather forecasting community, we were therefore somewhat surprised by the pessimistic ("bleak") interpretation given to our findings in Hollingsworth's *News and Views* article². Since this interpretation could be due to a misunderstanding, we would like to clarify our position.

By quantifying the sparseness of the meteorological observing network, we showed that no matter how large, phenomena sufficiently intense and sparse (with fractal dimension $D < 0.25$) would slip through the network undetected. The errors due to this limited dimensional resolution are more subtle than Hollingsworth seems to indicate. We implied neither that the network misses storms, nor the most energetic areas but rather, the most intense regions. Indeed, fundamental characteristics of the various levels of energy density (or more precisely, the flux of energy to smaller scales), are their multiple fractal dimensions which decrease as the intensity level increases. Since any set with $D < 1$ is a totally disconnected set of points, the regions missed, are the most active cores of storms. These low dimensional (sparse, core) regions play a crucial role in the future evolution of the atmosphere.

Hollingsworth seems to minimize the impact of this lack of dimensional resolution, first by citing the utility of existing forecasts 6–7 days ahead, second, by pointing to the increasing role of satellite data. Let us examine these points one by one.

Hollingsworth admits that even in the vague terms of "economical usefulness" forecasts are limited to periods of one week or less. Even without a precise discussion of predictability and its limits, is it unreasonable to suppose that at least part of our current difficulties are related to our inability to detect sparse but violent events?

Hollingsworth is obviously correct in pointing out the importance of satellite data. Indeed, since remotely sensed data generally have very high dimensional resolutions, our findings add a new argument in their favour. Unfortunately, the satellites themselves, are calibrated by sparse *in situ* networks, and current calibration methods do not recognize the problem of dimensional resolution. Furthermore, the existing four-dimensional data assimilation techniques that are used

to mix *in situ* and satellite data take no quantitative account of the various dimensions involved.

Perhaps, if we abandon the routine ways of dealing with the problem of sparse networks and phenomena, the future can be faced with optimism. New and mushrooming interest in techniques of multi (fractal)-dimensional analysis and simulation may ultimately help clarify basic problems in predictability. In the short term, we may expect rapid development of statistical techniques to provide important corrections to data lacking in dimensional resolution. Improved forecasts over a wide range of timescales could be possible.

SHAUN LOVEJOY
DANIEL SCHERTZER
PHILIP LADJOY

Physics Department,
McGill University,
3600 University Street,
Montreal, Quebec, Canada H3A 2T8

Météorologie Nationale,
2 Ave Rapp,
Paris 75007, France

1. Lovejoy, S., Schertzer, D. & Ladoy, P. *Nature* **319**, 43–44 (1986).
2. Hollingsworth, A. *Nature* **319**, 11–12 (1986).

Are arguments against archaeobacteria valid?

SIR—Until now we have commented only in passing on Lake's suggestions^{1,2} regarding archaeobacterial taxonomy and phylogeny, because their supporting evidence is weak. However, recent correspondence in *Nature*^{3–5} and attention elsewhere⁶ requires a stronger response.

The upper panel of Fig. 1 is a phylogenetic tree derived by a distance matrix-type analysis of the small subunit rRNAs. It shows the three primary kingdoms as distinct phylogenetic units⁷. (The same branching order results from parsimony analysis⁷.) In contrast, Lake's proposals (lower panel) distribute the various archaeobacteria among three separate 'kingdoms': the 'eocytes' (sulphur-dependent archaeobacteria, such as *Sulfolobus* and *Thermoproteus*), 'photocytes' (extreme halophiles and eubacteria) and methanogens^{1–3}.

Two issues are involved here, one scientific (the precise relationships of the other kingdoms to the archaeobacteria) and one semantic (whether Archaeobacteria is a proper taxon). The second issue distinguishes Lake's proposals from those of others, whose phylogenies assume archaeobacteria to be a valid taxon, although possibly paraphyletic⁷.

The factual basis for Lake's proposals is questionable. 'Eocytes' were defined as "a kingdom with a close relationship to

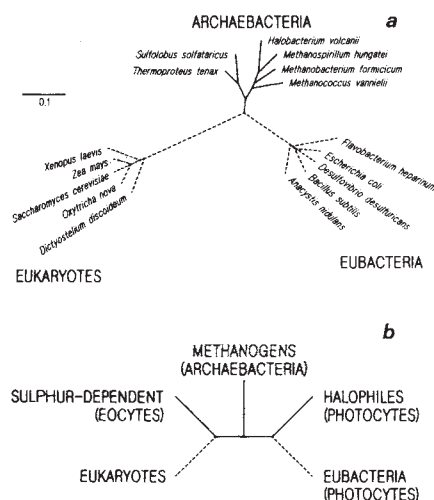


Fig. 1 The alternative views of archaeobacterial taxonomy.

eukaryotes" on the basis of the unusual shape of their 50S ribosomes, a shape ostensibly unique among archaeobacteria. However, a significant fraction of the 50S subunits in *Methanococcus vannielii* have this 'eocyte' shape⁸. Although what underlies these shape differences is not known, it is likely that they reflect relative protein content: subunits from both sulphur-dependent archaeobacteria and the *Methanococcales* have relatively large complements of protein compared with other archaeobacterial ribosomes⁹. Thus, dividing the archaeobacteria on the basis of ribosome type puts methanogens into two separate kingdoms — an untenable classification both phylogenetically and taxonomically.

'Photocytes' were defined on the basis of other perceived similarities in ribosome shape². In this case, too, the defining characteristics are not confined to the defined group⁸. Ribosome shape differences, which have never been shown to be homologous traits, are obviously not reliable determinants of major phylogenetic categories.

The proposals of Lake *et al.* have far too little supporting evidence, and much of this is of little or no phylogenetic value. Their argument rests heavily on the supposed absence of certain traits, which means little when it merely reflects a temporary failure to find them. For example, absence of introns outside the sulphur-dependent archaeobacteria was taken to be significant but introns were subsequently found in the extreme halophiles⁷. Ill-defined similarities, such as "common photosynthetic mechanisms", invoked in grouping eubacteria with the extreme halophiles, are more probably analogies than homologies: the (eu)bacterial chlorophylls have nothing in common with (halophile) bacteriorhodopsin, either structurally or functionally, whereas bacteriorhodopsin is structurally and functionally similar to eukaryotic rhodopsin —

hence its name. By invoking a small number of characters (especially ill-defined ones) one can construct many contradictory taxa: for example, cyanobacteria can be clustered with the extreme halophiles to the exclusion of other eubacteria on the basis of ferredoxin sequences; or, streptomycetes can be grouped with methanogens on the basis of their having coenzyme F420.

Lake's explanation of the rRNA-based tree (Fig. 1a) is that it must be an artefact of the treeing procedure (such procedures have a tendency to group rapidly evolving lines). Although such artefacts do occur, they obviously do not occur in every case involving unequal evolutionary rates. A thorough analysis indicates that the branching order in Fig. 1a does not reflect such an artefact⁷.

Returning to the semantic issue, regardless of whether the archaeobacteria are joined to the two other kingdoms by a single lineage (Fig. 1a) or by two separate lineages (Fig. 1b), archaeobacteria is a taxon of demonstrated predictive and organizational value⁷. It groups together prokaryotes with many characteristic phenotypic features, including composition of membranes, cell walls, rRNAs and tRNAs¹⁰. Lake's taxa, on the other hand, spread such organisms among several kingdoms (as indicated by the solid lines in the Fig. 1), intermixed with organisms not sharing their common characteristics. Whereas the grouping 'archaeobacteria' easily rationalizes almost all of what is known about these organisms, the Lake taxa are inconsistent with much of the phenotypic evidence and all of the sequence evidence bearing on the issue.

CARL R. WOESE

Department of Genetics and Development,
University of Illinois,
Urbana, Illinois 61801, USA

NORMAN R. PACE

GARY J. OLSEN

Department of Biology,
Indiana University,
Bloomington, Indiana 47405, USA

1. Lake, J.A., Henderson, E., Clark, M.W. & Oakes, M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3786 (1984).
2. Lake, J.A. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 3716 (1985).
3. Lake, J.A. *Nature* **319**, 626 (1986).
4. Zillig, W. *Nature* **320**, 220 (1986).
5. Lederer, H. *Nature* **320**, 220 (1986).
6. Wilson, A.C. *Scient. Am.* **253**(4), 164 (1985).
7. Woese, C.R. & Olsen, G.J. *System. Appl. Microbiol.* (in the press).
8. Stöffler-Meilicke, M., Böhme, C., Strobel, O., Böck, A. & Stöffler, G. *Science* **231**, 1306 (1986).
9. Cammarano, P., Teichner, A. & Londei, P. *System. Appl. Microbiol.* (in the press).
10. Woese, C.R. & Wolfe, R.S. (eds) *The Bacteria Archaeobacteria* Vol. 8 (Academic, New York, 1985).

Link between lamins and intermediate filaments

SIR—Recently McKeon *et al.*¹ reported in *Nature* an exciting result for cell biologists. Lamins, the major proteins of the nuclear envelope, show a striking sequence

and structural homology with the various proteins of the large family of cytoplasmic intermediate filaments (IFs). The complementary DNA sequence of lamins A and C predicts, however, an insert of 42 amino-acid residues in a coiled-coil domain highly conserved in length in all IF proteins (reviewed in ref. 2). Inspecting the reported lamin sequence I found that this insert starts precisely at a point of a common intron position previously found in several IF genes². The position of most introns of IF genes seems not to be related to the structural subdomains of the proteins². A similar situation was found also for myosin, another fibrous protein built from coiled-coils³. Thus, the position of the lamin insert is particularly interesting as it may indicate the evolutionary divergence between IF and lamin genes. This I expect to become more obvious once the entire lamin gene is known.

K. WEBER

Max-Planck Institute for
Biophysical Chemistry,
D-34 Goettingen, FRG

1. McKeon, F.D., Kirschner, M.W. & Caput, D. *Nature* **319**, 463-468 (1986).
2. Steinert, P.M., Steven, A.C. & Roop, D.R. *Cell* **42**, 411-419 (1985).
3. Karn, J., Brenner, S. & Barnett, L. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4253-4257 (1983).

The mechanics of visual acuity

SIR—Anyone who needs strongish (positive) reading glasses but does not suffer unduly from astigmatism can see that visual acuity is not just a function of eye lens quality plus receptor spacing. All that is needed is a red-light-emitting diode behind a square aperture. Looking at this without glasses produces *not* a single blurred image but an array of about eight red squares, more or less sharply defined. A 'perfect' eye would presumably produce a completely regular array; mine do not, and differ individually.

It follows that the eye-cortex combination acts holographically, in that separate portions of a blurred image give rise to the impression of a sharp image. Furthermore, if instead the image of a point source is properly focused, the system can be expected to give rise to a visual acuity greater than indicated by optical considerations alone.

The compound eyes of insects are known to act in a similar manner, because individual images are too poor to account for bees' discriminatory power, for example. So it is perhaps not surprising that the larger animal eye, producing good optical images, should nevertheless have retained the image-processing power of the cortex representing an earlier stage in the evolution of the eye.

T. NASH

Bower Chalke,
Salisbury, Wilts SP5 5BW, UK

The human factor in mammoth extinction

SIR—A recent *News and Views* piece by Diamond¹ discusses mammoth hunting and extinction. While I agree that sophisticated hunting techniques must have been available to palaeolithic mammoth hunters, close spearing or hamstringing, as practised by modern Pygmy elephant hunters, is dependent on the possession of razor-sharp iron spearheads²; and the existence of a pharmacopoeia of poisons among Palaeolithic hunters is of course undocumented. Trunk snares or weighted hanging spears would seem to depend on the prey moving among rather heavy forest vegetation, whereas the stomach contents of mammoths attest to their inhabiting sparsely forested, open, steppe-like grasslands. Palaeolithic peoples probably trapped mammoths with fire or with footsnare and pitfalls set or dug along regularly used routes. As zoo curators know, a fall of only a metre or even a stumble may be potentially fatal to a large mammal, so a footsnare or pitfall would have been a relatively safe and effective aid to mammoth hunting.

Nevertheless, contrary to Diamond's contention, French and Spanish cave paintings do not unequivocally show these hunting techniques. Tectiform signs in Palaeolithic art are certainly sometimes interpreted as depicting traps and pitfalls, but the interpretation of these signs is subject to debate. They have also been viewed as depicting shelters or maps, or as having a more esoteric symbolic significance.

Finally, Diamond concludes that mammoth extinction is probably the direct result of the proficiency of human hunting. However, the most recent compendium of explanations³ for Pleistocene megafauna extinctions appears to favour ecological or climatic causes. Certainly the Pleistocene overkill hypothesis originally elaborated by P.S. Martin has not been accepted without debate, and may have lost some potency as an explanatory hypothesis during the past twenty years, even with the introduction of the concept of a blitzkrieg overkill which might explain the paucity of archaeological evidence.

SUSAN CACHÉL

Department of Anthropology,
Douglas College, Rutgers University,
New Brunswick, New Jersey 08903, USA

1. Diamond, J. *Nature* **319**, 265-266 (1986).
2. Coon, C.S. *The Hunting Peoples* (Little, Brown, Boston, 1971).
3. Martin, P.S. & Klein, R.G. *Quaternary Extinctions* (University of Arizona Press, Tucson, 1984).

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published.

What's wrong with nuclear power?

David Pearce

Nuclear Politics: The History of Nuclear Power in Britain. By Tony Hall.
Pelican: 1986. Pp. 200. Pbk £3.95.

THE history of the civilian nuclear power industry in Britain is one of concealment, dubious promises, a reckless approach to technology and naive public relations. The concealment has shown in the attempts to hide the poor safety record of the industry's centrepiece, Sellafield; the dubious promise is revealed in the debatable nature of the comparative economics of nuclear electricity generation; and the recklessness was underlined nearly ten years ago by the Flowers Commission's insistence that waste disposal problems should be solved before, and not after, further expansion of nuclear capacity.

It is, however, in its public relations that the nuclear power industry has made its worst mistakes. With arrogance compounded by naivety the authorities have consistently misread public opinion, with the result that if support has not been forthcoming the public has been treated to a further dose of "education". Such an educational programme demands novel and sympathetic approaches to social acceptability, but the industry is dominated by technologists whose training makes them unequal to the task. The process of decline feeds on this acceptability gap: nuclear power is not just about scientific solutions to scientific problems, it is about the social status of scientists and technologists thrust awkwardly into the political firing line.

It requires only a recent episode to illustrate this lemming-like syndrome. With the accidental discharge of uranium nitrate into the Irish Sea, and admission by the authorities of a separate, internal incident involving plutonium, a newly formed Nuclear Electricity Information Group (itself part of the syndrome — the industry invariably alters titles and names when on the retreat) issued its first advertisements. In one of these, three pub-goers, one looking remarkably like the archetypal university student, reveal that what the common people think of nuclear power is that reactors manufacture acid rain and nuclear electricity produces radioactive light bulbs. Either the advertising agency, and perhaps NEIG, believe these are common misconceptions or the advertisement is a nasty attempt to set up a straw man image of the opposition to nuclear power. Whatever the case, it speaks volumes for the lack of understanding in the nuclear industry of social processes.

Therein lies the clue both to what has gone wrong for nuclear power and

perhaps to the industry's last chance to secure a degree of respectability, at least among the limited groups remaining who are prepared to be even-handed. No information packs for schools, crass advertisements or new shops in towns adjacent to potential waste dumps will substitute for the necessary self-criticism that would arise from asking *why* opposition to nuclear expansion is so fierce and articulate. The days of the early smear campaigns when opponents were branded as Soviet dupes are over. Serious questioning of the fundamental social rationale for nuclear power has found its way into Select Committees, the Monopolies and Mergers Commission, the Royal Commission on Environmental Pollution and many other bodies which cannot be dismissed in the traditional way.

In *Nuclear Politics*, Tony Hall touches on some of the reasons for the focusing of dissent. He is especially detailed and robust on the early years: the links between nuclear power and nuclear weapons, the paranoid secrecy and the distance the industry managed to secure from political control (this being as much due to the ignorance of the politicians as to the propaganda they swallowed). When environmentalism finally became not just a popular but also a respectable political force, the industry had neither the tradition nor the capabilities to handle it. How different the picture might have been if the industry had understood the public perception of risk is hard to say; the mistakes and cover-ups would still have happened, but the effects on public confi-

dence would surely have been less. Instead, the industry reacted in the technological mode. For instance, it persisted with the definition of risk as the probability of death or morbidity, and ignored the concepts rooted in social science which emphasize the nature of both the event and the process leading to the event, as well as the probability. The apotheosis of error in the technological approach to risk was revealed in Lord Rothschild's famous Dimbleby Lecture, warmly applauded by the industry at the time and rightly derided by anyone with a smattering of social understanding. Sadly, Rothschild's thinking still pervades the industry, once again a sad reflection of the absence of a social-science base to pro-nuclear politics.

Tony Hall takes us from the early days to the development of the Magnox programme and on to the AGR "disaster", though strangely omitting any reference here to the damning work of David Henderson in 1977 on the economics, or lack of them, in the AGR programme (a critique which he repeated in last year's Reith Lectures). Hall then outlines the emergence of the opposition to nuclear power, notably the Friends of the Earth and the intellectual spur to respectable criticism from Amory Lovins and Walter Patterson. Missing in this account, however, is mention of the emergence of many doubters in the economics profession — it was never easy to find academic support for the nuclear case, for example. Hall then outlines the famous Windscale Inquiry into the planned new processing plant, now under construction (and under Parliamentary scrutiny). The nuclear industry again misjudged the public view of matters when it greeted so positively the extraordinary bias and eccentricities in the report of the Inquiry. It was almost as if any support would do, regardless of its quality.

Nuclear Politics is at its weakest in its concluding sections on Sizewell. There is

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

"Everyone's got an opinion. But who's got the facts?" Nuclear Electricity Information Group advertisement in *The Guardian*, 3 February 1986.

no summary of the arguments or analysis of the inquiry process, nor any reflection on the attempt to force the demise of the "big" inquiry by the present government, something the industry has always wanted. This is a shame because Tony Hall writes with admirable clarity and skill. The layman's guide to Sizewell will need to be written, while specialists must await Professor O'Riordan's University of East Anglia/Economic and Social Research Council study for the definitive analysis. The other area still to be documented is the role of the regulatory agencies in the fortunes of nuclear power: the Nuclear Installations Inspectorate, the National Radiological Protection Board and the Health and Safety Executive do not have a prominent place in the book.

Nonetheless, Tony Hall has brought together an accurate yet readable mix of the technology and politics of nuclear power in Britain. The pity is that those who need to take on board its lessons will, more likely than not, again fail to do so. □

David Pearce is a Professor in the Department of Economics, University College London, Gower Street, London WC1E 6BT, UK. He is co-author of Decision Making for Energy Futures: A Case Study of the Windscale Enquiry (Macmillan, London, 1979), and is a member of the National Radiological Protection Board (NRPB). The opinions expressed in the review are personal and should not be taken to reflect those of the NRPB.

This is a great book,
and an exciting book...
readable, worth reading
and enlightening.

Sir Karl Popper

Cosmology, Physics & Philosophy by Prof Ben Gal-Or

"a master piece... any good
library must have a copy of this
classical work."

Ind. J. Phys.

"... a tour de force... ill be widely
read and appreciated... a mag-
nificent and sustained piece of
work!"

Sir Alen Cottrell

... remarkable... challenging...
Am. J. Phys.

2nd Printing
Springer-Verlag
175 5th Ave., N.Y., 10010

Rich cuttings from a medical life

Michael Shortland

William Hunter and the Eighteenth-Century Medical World. Edited by W.F. Bynum and Roy Porter. *Cambridge University Press: 1985. Pp. 424. £35, \$49.50.*

FOR MUCH of its existence, medicine has been the most disreputable of the learned professions — less conspicuous than the law or the church, but its failings for that reason all the more evident. The shady lawyer could lose you a case, the venal priest would sell you a soul, but a visit to your physician might cost you an arm and a leg, and quite possibly your life. Until anaesthetics and antiseptics were developed, the best medicine was no medicine. As Swift put it, the only doctors to be trusted were Doctor Diet and Doctor Quiet.

The defects of medicine were never more visible than during the eighteenth century. Pilloried in the press, ridiculed by satirists and subject to scandal, the physician seemed for a while ready to close shop and hand the keys over to the charlatan pill-pushers who reaped vast profits from the poor soil of conventional physic. These itinerant quacks had one crucial fact in their favour: they might fail to cure your disease, but they did not kill you with their remedies. Early in the century, two rival physicians, Drs John Woodward and Richard Mead met in a duel; supposedly, Mead disarmed his opponent and humanely exclaimed "Take your life". To which Woodward replied "Anything but your potions".

Such an episode may not have been typical, but it was symptomatic. With so few accepted standards of health care and no organization able to police the profession, disputes and disarray were endemic. Lacking a strong self-identity, the doctor found himself aligned, then harangued, with the barber-surgeon and the grocer-apothecary. Moreover, as the second editor of this volume explains, at a time when religion indoctrinated people with a profound belief in the corruptions of the flesh, medicine suffered from a guilt by association. Particularly in branches such as venereology and obstetrics, the humble practitioner was liable to find himself stigmatized by vice and sexual impropriety.

How curious then that William Hunter (1718–1783), whose father intended him for the ministry, should opt instead for a life in medicine. Strange, too, that after a surgical training in provincial Hamilton and then in London, he should gravitate towards obstetrics. But truly astonishing is the discovery, detailed with evident relish

in this collection of essays, that Hunter managed to acquire such eminence and wealth pursuing this doubly-notorious trade. As Porter explains in a brilliant assessment of this apparent misnomer, the gentleman-surgeon, Hunter had to start life in the wrong country (Scotland), in the wrong religion (Presbyterianism) and in the wrong family (large and rough-cut).

Good luck along the way no doubt helped Hunter reach the heights of his adopted profession. But as important was his keen sense of what his potential customers required. From the contributions here on apprenticeship and medical education in Britain and on the continent, it is clear that Hunter made good the relatively dismal opportunities for training by offering private anatomical classes in London hospitals. These were marketed with the same skills he brought to the surgery: precision, efficiency and style. Knowing the opprobrium that attached itself to his line of work, Hunter carefully cultivated contacts and clients. The job of an obstetrician demanded discretion, so he kept a low profile. But he could be showy, exhibitionist and highly visible in different circumstances. In an age when appearances mattered, Hunter could adopt many — in every sphere his skills as a practitioner were enhanced by formidable entrepreneurial acumen.

This is not to belittle his contribution to medical knowledge, though in that domain William's labours were eclipsed by those of his brother John, the famous physiologist and surgeon buried in Westminster Abbey and commemorated by the Hunterian Society. As the final section of this book reveals, William was an innovative and influential male midwife, and a non-interventionist at a time when many of his colleagues saw the recently-designed forceps as the key to the lying-in room.

In this aspect of his career as in others it remains difficult to see whether we are dealing with a remarkable exception to the rule that eighteenth-century medicine was primitive and squalid. It may well be that we should instead view William Hunter as part of the evidence in a general revision of the history of medicine. Certainly, this rich and engrossing volume offers insights into a world very different to that portrayed by Victorian doctors who enjoyed dating all that was proper in medicine to their own time. Whether or not William Hunter fits that particular portrayal is a question which the contributors here tackle from different perspectives and with great subtlety, but the question remains hanging in a promising state of uncertainty. □

Michael Shortland is in the Department for External Studies, University of Oxford, Rewley House, 1 Wellington Square, Oxford OX1 2JA, UK.

At the frontiers in South America

Norman Myers

Amazonia. Edited by Ghilleen T. Prance and Thomas E. Lovejoy. Pergamon: 1985. Pp. 442. £19.50, \$27.95.

A DOZEN or so scientific books dealing with Amazonia have appeared since 1980. This upsurge in professional interest is heartening, because it reflects a new awareness on the part of the scientific community both within Amazonian nations and elsewhere. After all, there is much to be aware about: with the richest biotic communities on Earth (one fifth of all species), Amazonia represents a kind of frontier for modern biology. Yet we know all too little about what makes it tick, let alone how to keep it ticking.

The region is also a frontier of different sort. The eight nations concerned want to develop it; that is, to exploit it, populate it, do *something* with it, rather than just leave it "idle" and unoccupied. Lacking science-based models of development, they have tended to employ approaches that owe more to notions of temperate-zone development than to an appreciation of what Amazonia has to offer. Fortunately there are signs of a change in attitude, as governments and international agencies explore the creative challenges of a new frontier in the dual senses.

For all the outburst of writings, no book has reflected the intrinsic complexity of the region, whether seen from the standpoint of the scientist or the developer. Virtually all the material hitherto available is descriptive rather than analytical in style, and traditionally academic in spirit. How pleasing, then, to come across this book, a volume in Pergamon's *Key Environments* series, with its emphasis on relationships and interactions — and not only between biotas and their environs, and so on, but between scientific disciplines with all that should imply for coordination between government departments. The chapter on fish, for instance, reveals not only that Amazonia possesses 3,000 species (15 times as many as Europe), and that commercial fisheries could produce far more animal protein than do the region's cattle ranches; more to the point, we read about entire fish communities, including the 50 species that generate three-quarters of the commercial harvest, and that feed primarily off fruit fallen into the water. This bodes much for plans to turn the alluvial floodplains into rice fields by eliminating riverside forests.

A similar approach characterizes the chapters on soils, vegetation, agriculture, forestry, and useful plants and animals,

among others. The most important chapter of all, however, is probably the one on climate. It shows how the Amazonian forest makes some of its own precipitation, and thus illustrates the ultimately integrative nature of the biospheric sciences; that is, life is not only modified by its environments but it adapts them as well, often to its own long-term benefit. This means that deforestation of part of Amazonia would reduce rainfall for the rest of the region, and thus alter the entire character of the remainder of the forest no matter how well protected it might be. It further implies that rainfall patterns would be affected in southern Brazil as well, this being the country's main crop-growing zone.

So here is a book to applaud. Within its 175,000 words, it encompasses the main components of the great Amazonian ecosystem. Several of the chapters are by Latin Americans, another welcome departure because most scientists interested in the region are still located in North America. My main regret is that there is not more text by the editors. Their two-page Introduction is splendid stuff: all the more pity, then, that they did not include commentaries on each chapter, or at least on the three main sections, with a concluding overview to pull the whole thing together. □

Norman Myers, Upper Meadow, Old Road, Headington, Oxford OX3 8SZ, UK, is a consultant on development and environmental matters.

Insider's insects

Timothy J. Bradley

Fundamentals of Insect Physiology. Edited by Murray S. Blum. Wiley: 1985. Pp. 598. \$39.95, £41.40.

DESPITE the number and variety of biology students taking classes on insect physiology, we have hitherto lacked a comprehensive, detailed and up-to-date textbook on the subject. *Fundamentals of Insect Physiology* is a successful attempt to meet that need.

The book contains chapters on most of the traditional areas of physiology, including respiration, circulation, endocrinology, exocrine glands, and metabolism and behaviour, as well as discussion of topics related to neural and muscular function. Both its strengths and its weaknesses derive from the multi-author format. The information is in most part well presented and amazingly free of factual error, as is to be expected when experts are writing about their own specializations. Conversely, while the stylistic differences are not serious, the clarity of writing differs widely and there is little coordination between contributions on related topics.

Some of the chapters will unfortunately be of little use to beginning students of insect physiology. The lengthy account of electrical events in the nervous system is marred by confusing and poorly printed figures, and the examples are drawn largely from experiments using molluscan and mammalian material. More concise explanations of action and receptor potentials are available in basic physiology texts. The chapter on intermediary metabolism presents most of the metabolic pathways as confusing lists of reactions, rather than as pathways connected with arrows; for this topic, students would better be directed to *Insect Physiology* by Mordue *et al.* (Blackwell Scientific, 1980), an excellent but much less comprehensive textbook.

Most of the authors, however, present accurate and readable accounts of their fields. One of the many excellent contributions is the chapter on respiration by T.J. Nation. After a detailed description of morphological adaptations, both in body plan and at the organ and cellular levels, the author proceeds to a mathematical discussion of the physical laws underlying the diffusion of gases, ventilation and the metabolic rate of insects in various physiological states. This is followed by an analysis of specific respiratory adaptations (in pupae, aquatic insects and eggs), with examples of species possessing each type of adaptation. This last feature will be helpful for students faced with the bewildering morphological and taxonomic diversity of insects, for example among those possessing tracheal gills. All of the chapters contain references to review articles and sometimes to the primary literature.

The sequence of material could have been better; for example a discussion of the effects of hormones on behaviour precedes the chapter on endocrinology. Worse, however, is the choice of the opening chapter. My heart goes out to the students who, using this book, begin their acquaintance with insect physiology by reading a rather opaque discussion of the embryology of the insect heart.

The dust jacket indicates that the book is intended "both as a text for courses in insect physiology and as a basic reference for entomologists, zoologists, pest managers and physiologists". We already have an abundance of material in the second role, and it is as a basic text that *Fundamentals of Insect Physiology* has the most to offer. One hopes that later editions will continue to emphasize this aspect. Even as it stands, however, I consider it to be the best such textbook available. □

Timothy J. Bradley is in the Department of Developmental and Cell Biology, University of California at Irvine, Irvine, California 92717, USA.

Between conservation and catches

T.J. Pitcher

Marine Mammals and Fisheries. Edited by J.R. Beddington, R.J.H. Beverton and D.M. Lavigne. *Allen & Unwin*: 1985. Pp.354. £40, \$55.

INVASION of Earth by powerful, opportunistic and intelligent competitors for its fishery resources would immediately bring calls for their extermination. Yet in this role of conflict with our own species we already have marine mammals, native creatures evolved as part of the delicate balance of ocean ecosystems. Can the management and conservation of marine mammals, many of which by virtue of recent exploitation are rare and endangered species, be reconciled with the optimal control of fisheries? The 20 papers in this timely, readable and well-edited volume arose from a 1981 workshop in La Jolla which addressed the scientific basis of the question, mainly for seals and whales.

Two of the founders of quantitative resource assessment, Sidney Holt in a foreword, and Ray Beverton in Chapter 1, introduce the key issues. Marine mammals may affect catches by eating fish and squid; they may act as intermediate hosts to economically damaging parasitic nematode worms in fish; and they themselves may become lethally entangled in, and damage, fishing gear. Fishery/mammal conflict often brews up despite inconclusive evidence one way or the other, but it is difficult to produce unequivocal scientific analyses because our understanding of the population dynamics of marine mammals is limited: the complex social behaviour of marine mammals makes reproductive and feeding rates hard to predict. Holt considers that this book sets out "where we now are" in this field. I agree, and it does so with admirable clarity.

Beverton looks to technology to solve the entanglement problem, and sets the scene for many of the case studies later in the book. His main contribution, however, is cleverly to modify conventional fishery equations to show how fish stocks are affected by various levels of control imposed on the mammal population, and vice versa. Where the mammal is itself not exploited, his conclusion is that there is no optimal solution allowing coexistence and so the trade-off between fish yields and mammal conservation must be decided using other criteria. Beverton's analysis offers predictions of the consequences of decisions, but not the means for making them.

This theme is taken up in the second chapter by Colin Clark, a leading analyst of resource economics, who concurs that no conventional optimal management criteria favour mammal/fishery coexistence. Clark searches for a pragmatic solution reminiscent of John Pope's "minimum sustainable whinge" fishery strategy, which aims to produce the least dissatisfaction among the interest groups concerned. Maintenance of ecosystem integrity is also one of Clark's goals, although I wonder if ecologists could ever agree how to measure it.

Some time ago, Clark demonstrated how strongly-shoaling fish such as mackerel are easily overexploited because fishermen maintain good catches right up to the last shoal in the sea. Likewise, it pays marine mammals to prey upon these profitable food patches, exacerbating depletion of both mammal and fish populations. Clark advocates careful spatial partitioning of fishery quotas as the only solution.

Both of these opening chapters covering fundamental issues are stimulating and challenging. Twelve case studies, ranging geographically from California to Kerguelen, follow, and although they inevitably vary in approach the net is cast sufficiently wide for the general reader to find something of interest. Three chapters

particularly caught my attention.

Beddington and de la Mare critically describe models aimed at limiting the impact of Southern Ocean krill fisheries on the recovery of Antarctic whales. The role of such strategic simulation models is seen as guiding the acquisition of relevant information, thereby producing insight rather than mere data. Such laudable goals for ecological modelling are rarely achieved and the perceptive review in this chapter could help us get there more often.

Harwood and Greenwood examine dispassionately and in detail the scientific basis of the recent controversial government cull of Scottish grey seals: this chapter will be essential reading for conservationists. The cull had unanticipated and damaging consequences for the seals. Although killing them should increase fish catches, it has so far proved impossible accurately to quantify this effect, whether in numbers, weights or economic terms.

When pole and line capture for tuna changed to mechanized purse seining in the 1950s, dolphins often associated (for unknown reasons) with tuna schools began to be slaughtered in large numbers. Allen discusses the East Pacific yellowfin tuna fishery where one dolphin dies for every three tonnes of tuna landed. He analyses the costs of concentrating on tuna schools not accompanied by dolphins.

The final section of the book contains six reviews of methods of estimating fish consumption by marine mammals. These, I think, would have been more appropriately published in the specialized literature.

Marine Mammals and Fisheries will be indispensable for biologists working on the management of marine resources. It should also attract a wider readership among those concerned to resolve conflicts arising from our exploitation of other species with which we share this planet. □

T.J. Pitcher is a Senior Lecturer in the School of Animal Biology, University College of North Wales, Bangor, Gwynedd LL57 2UW, UK.

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Detail of a model of the Cap-Ferret submarine fan, a complex sedimentary body located in the south-eastern part of the Bay of Biscay. (Bathymetric curves are 50m equidistant, scale is c. 12.25km cm⁻¹; the numbers refer to features identified in the original.) The illustration is taken from Submarine Fans and Related Turbidite Systems, the first volume in a new series, Frontiers in Sedimentary Geology, published by Springer-Verlag. Editors of the book are A.H. Bouma, W.R. Normark and N.E. Barnes, price is DM 220.

Screwworm eradication a grand delusion?

from J. L. Readshaw

A campaign to eradicate the screwworm, a major pest of livestock in Texas, has cost \$50 million a year. But the claimed success of the programme can be explained by natural variation and new outbreaks may well occur when climatic conditions again favour the pest.

THE campaign to eradicate screwworm, *Cochliomyia hominivorax* (Coquerel), from the southern United States is the most ambitious entomological programme ever mounted. Its progress over the past 26 years has spawned similar multi-million dollar programmes, aimed at eradicating other major insect pests throughout the world¹.

The screwworm is a primary parasite of livestock, a tropical species, distributed throughout central and south America and the Caribbean. It extends northward through Mexico and southern Texas, and occasionally spreads throughout southern and central United States causing serious losses to livestock and wildlife².

The adult fly lays batches of eggs on the edges of open wounds. The resulting 'screw-like' maggots burrow into the living flesh, causing myiasis, weakening the animal and occasionally causing death. When mature the maggots leave the wound, drop to the ground, and pupate in the soil—producing adult flies some 10 to 20 days later.

Several generations occur during the warmer months, but the fly has no dormant or protected overwintering stage and thus cannot survive in areas where the average winter temperature drops below about 10 °C (50 °F). Thus the fly normally overwinters only in the extreme south of the United States but reinvades the northern States in spring and summer—the adults being highly mobile.

The eradication programme was conceived by E. F. Knippling in 1938³. It is based on the release of millions of artificially-reared sterile males. Male sterility is induced by exposing 5-day-old pupae to γ rays. Because of their superior numbers, the sterile males outcompete the wild males in mating with the wild females. As a result, the wild females lay only sterile eggs; no offspring are produced, and, in theory, the population dwindles to extinction⁴.

The research programme started in 1950, with X-ray and later γ -ray induced sterility⁵. Field trials on the islands of Sanibel and Captiva, off the west coast of Florida, showed promise (G. W. Eddy, A. H. Baumhover, A. J. Graham and F. H. Dudley, unpublished data quotation ref. 6) and, in 1954, a major campaign on Curacao was judged a success⁷. Another

large-scale trial in Florida in 1957⁸ provided the impetus for a massive campaign to eradicate screwworm from peninsula Florida, starting in January 1958 and ending with the eradication of screwworm from all the south eastern States by November 1959^{9,10}.

Thereafter (1962 to the present) the programme was taken to the south-west states, mainly Texas, New Mexico and Arizona, with a major extension into Mexico in 1976, involving the opening of a new sterile-fly factory at Tuxtla in 1981¹¹.

Meanwhile, the 'sterile insect release method' (SIRM) was used to eradicate screwworm from Puerto Rico and the Virgin Islands (1974–75)^{12,13}, and to re-eradicate it from Curacao (1977)¹⁴. Further campaigns are now under consideration for Trinidad and Tobago¹⁵.

Flimsy evidence

The scientific basis for screwworm eradication using sterile males rests on only two publications—the first covering the Curacao campaign which lasted less than five months⁷, and the second the Florida trial⁸, which lasted only three months before being overtaken by the Florida campaign. Except for more recent work in Mexico^{16,17}, no other systematic data on population levels or sterility have been published. References 7 and 8 are quoted repeatedly in the literature, yet their relevant content occupies only two small tables. Moreover, 'success' of the various campaigns is now accepted as established 'fact'. No cases of screwworm have been reported in Florida since 1972 and only very few in Texas since 1978.

But a recent manuscript by Krafur¹⁸ raises the possibility that screwworm is influenced by climate. This report presents an analysis of the incidence of screwworm cases in Texas in relation to seasonal temperature and moisture conditions during the 1962–82 south-west eradication campaign.

Historical perspective

Records of screwworm in the United States go back to 1832². In the 1890's it was a serious problem in Texas and the Gulf Coast and subsequently there have been periodic outbreaks, particularly from 1932 to 1935 when massive infestations developed, reaching Florida and the south

eastern States in 1933¹⁹ and extending up to the Great Lakes in 1935.

The progression of this outbreak is interesting. Before early summer 1933, screwworms were not known in Florida, nor in any of the southeastern states. In Florida, the first cases appeared in the extreme north of the state, near Georgia¹⁹. Earlier, in October 1932, screwworm was common but mild in Mississippi¹⁹; in 1933 it was present in southern Alabama²⁰ and Georgia²¹; in 1934 it was a serious problem from the Texas gulf coast, through Louisiana²², Mississippi¹⁹, southern Alabama²⁰, Georgia²³ and northern Florida²⁴.

This pattern of spread suggests that the screwworm probably reached Florida

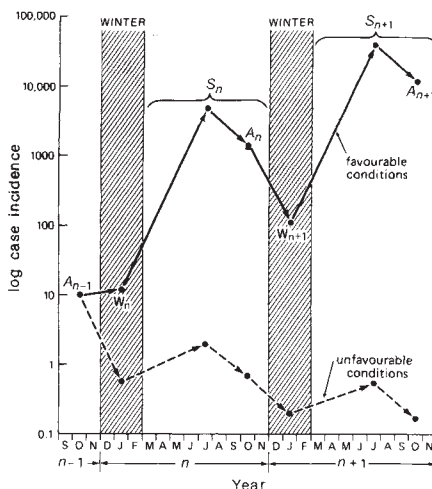


Fig. 1 Hypothetical model illustrating screwworm increase in relation to favourable or unfavourable seasonal conditions.

naturally by eastward movement from Texas and Louisiana during 1932–33, not, as is often claimed, imported on infested stock⁶. In fact Dove and Parman specifically say that the cases in northern Florida occurred *before* the importation of livestock from the drought-stricken western states.

The 1933–35 outbreaks ended throughout the southern States following the very cold winter of 1935–36. The next reports are for 1943, with losses in both the southwestern and southeastern states, following the mild winter of 1942–43. Thereafter, serious outbreaks occurred in association with mild winters in 1945, 1948

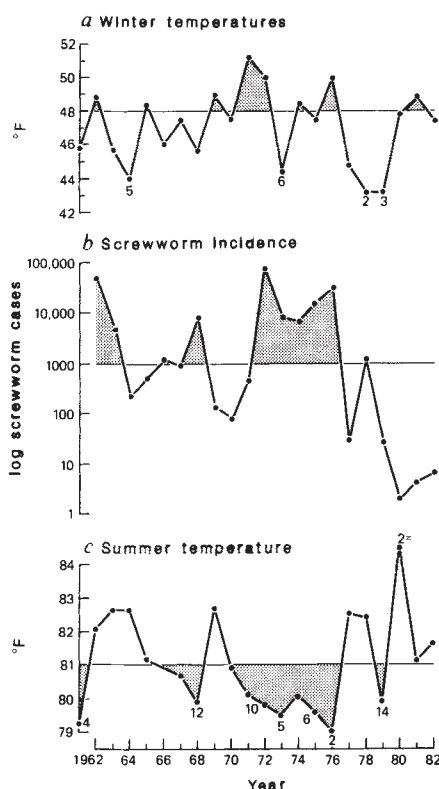


Fig. 2 Correspondence between screwworm case incidence (b), winter temperatures (a) and summer temperatures (c) in Texas between 1962 and 1982. The numbers indicate rank with respect to highest or lowest temperatures during the past 100 years.

and 1949 right across the southern United States²⁵. In Florida, another cold winter occurred in 1950–51, “bringing screwworm activity to an end”²⁵, but for how long the populations remained low is unclear.

The Florida trial occurred during an outbreak in 1957, following the mild 1956–57 winter, but the eradication campaign was accompanied by the coldest Florida winter on record (records started in 1891)²⁶. Similarly, the eradication campaign in the southwest states, starting in 1962, was fortunate in coinciding with two successive cold winters (1962–63 and 1963–64).

During the south-west release programme, outbreaks occurred in 1968 and from 1972 to 1976, with an estimated peak of 2 million cases (equivalent to 29,000 reported cases) in Texas in 1976²⁷. Incidentally, two cases were reported from Florida in 1972, but none has been reported there since.

During the past five years there have been very few cases of screwworm anywhere in the United States. Eradication is claimed¹¹ and the programme has moved into Mexico to create a barrier zone. But eradication has been claimed before: for Texas and New Mexico in 1964, and Arizona and California in 1965²⁸.

Thus, it is prudent to ask to what extent is the alleged success of the screwworm

campaign due simply to unfavourable (for the fly) seasonal conditions?

Texas

The numbers of confirmed cases of screwworms in Texas during the course of the eradication programme are listed in monthly reports of the Texas Agricultural Extension Service²⁷. Given these data the following hypothesis can be tested.

The seasonal incidence of screwworm cases in Texas during the eradication campaign was determined in part by: (1) winter temperature, influencing winter survival, and (2) summer temperature and moisture influencing seasonal breeding success.

The hypothesis is illustrated in Fig. 1, with winter cases in year n (W_n) being derived from the previous autumn's cases (A_{n-1}), according to winter temperature conditions (wt_n); and with seasonal cases in year n (S_n = spring + summer + autumn cases) being derived from winter cases (W_n), according to summer temperatures (st_n) and summer moisture (sm_n) conditions. The relevant climatic variables for autumn (September–November), winter (December–February) and summer (June–August) for the State of Texas²⁶ are listed in Table 1, together with the release rates of sterile males during the Texas programme. Also shown is the seasonal incidence of screwworm, which, because of the delay in reporting cases, is expressed on a quarterly basis (winter cases W_n = January–March and so on).

Adding one to the case data and transforming to natural logarithms, the hypothesis may be expressed by two linear equations:

$$\ln(W_n + 1) = a + b \ln(A_{n-1} + 1) + cwt_n \quad (1)$$

and

$$\ln(S_n + 1) = d + e \ln(W_n + 1) + fst_n + gsm_n \quad (2)$$

where W , A and S are numbers of screwworm cases reported in years n or $n-1$ and a , b , c , d , e , f , g are coefficients to be estimated by multiple regression. The analysis shows that the equations

$$\ln(W_n + 1) = -16.24 + 0.45 \ln(A_{n-1} + 1) + 0.34 wt_n \quad (3)$$

and

$$\ln(S_n + 1) = 69.25 + 0.96 \ln(W_n + 1) - 0.80 st_n \quad (4)$$

estimate the actual incidence of winter cases (W_n) and seasonal cases (S_n) with an accuracy of 74% and 67% respectively (Table 2).

The influence of winter temperature was highly significant ($c = 0.342 \pm 0.101$, $t = 3.38$, $P < 0.01$); summer temperature less so ($f = -0.803 \pm 0.316$, $t = 2.54$, $P < 0.02$); whereas summer moisture, expressed as

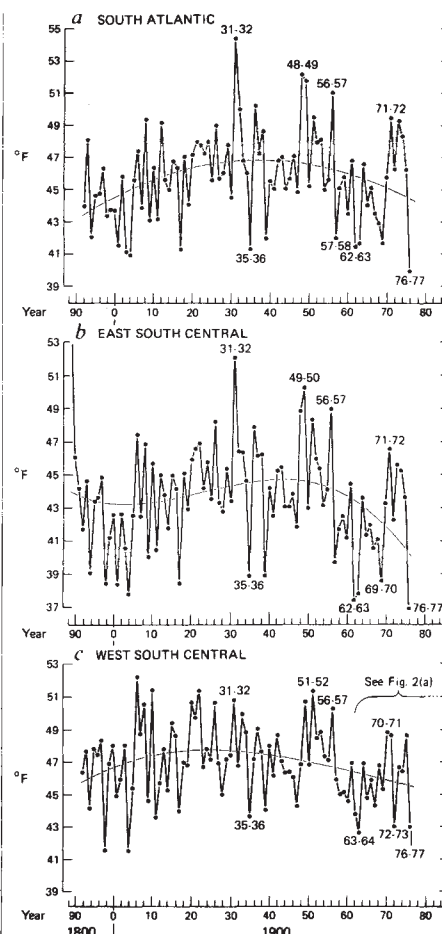


Fig. 3 Long-term trends in winter temperatures (December–February) since the late 1800s in three contiguous climatic regions of the southern United States. The trend lines are third-order polynomial regression curves. See ref. 29 for more detail and regional boundaries.

either rainfall or the Palmer Drought Severity Index (PDSI) was not significant. However, summer temperature and moisture are negatively correlated such that it is necessary only to include temperature in equation (4).

In other words, given the numbers of autumn cases in Texas in year $n-1$, we can estimate the subsequent numbers of winter cases simply on the basis of winter temperature. Similarly, starting with winter cases, we can estimate the subsequent numbers of seasonal cases on the basis of summer temperature.

The inclusion of sterile male release rates in the model gives an interesting though clearly spurious result. It actually increases the amount of variation accounted for by the model to about $r^2 = 0.82$ in the case of predicting winter cases from autumn cases, and to $r^2 = 0.76$ in predicting seasonal cases from winter cases. But in each case the prediction is in the wrong direction as far as the programme's objectives are concerned. The inclusion of release rates gives *positive* rather than *negative* regression coefficients equal to +0.245 and +0.403 respectively. This sug-

Table 1 Incidence of screwworm cases in Texas in relation to sterile male release rates and seasonal climatic conditions

Year	Screwworm cases in:			Release rates $\times 10^6$ per day	Winter temperature (°F)	Summer temperature (°F)	Summer rainfall (inches)	PDSI*
	Autumn (A_{n-1})	Winter (W_n)	Season (S_n)					
1962	?	?	49,363	2.6	47.7	82.1	7.86	?
1963	12,470	217	4,699	6.9	45.7	82.7	5.89	-4.10
1964	2,628	7	216	1.8	44.0	82.7	5.54	-4.04
1965	124	4	462	1.0	48.3	81.2	5.60	-1.72
1966	144	23	1,180	1.7	46.0	80.9	8.54	0.50
1967	855	3	832	2.0	47.4	80.7	6.65	-3.93
1968	551	2	9,266	3.8	45.7	79.9	9.14	3.64
1969	5,577	26	135	2.6	48.8	82.7	5.85	2.05
1970	4	0	92	1.8	47.5	80.9	4.68	-0.54
1971	8	8	436	2.5	51.2	80.2	10.07	-4.34
1972	217	115	90,865	8.4	50.0	79.8	8.73	0.02
1973	19,329	59	8,854	6.4	44.4	79.5	10.60	3.57
1974	5,480	106	6,796	7.9	48.5	80.2	8.82	-2.34
1975	3,321	31	16,733	7.8	47.6	79.6	8.89	4.08
1976	7,383	225	29,016	9.2	50.0	79.0	8.96	1.58
1977	9,266	6	33	6.0	44.8	82.5	5.93	-0.80
1978	4	0	1,236	4.1	43.2	82.4	7.23	-3.40
1979	419	2	30	1.0	43.2	79.9	10.28	3.23
1980	0	0	2	?	47.9	84.5	4.46	-1.76
1981	0	1	4	?	48.8	81.1	11.00	1.54
1982	0	0	6	?	47.4	81.7	6.81	1.98
1983	0	0	0	?	?	?	?	?
1984	0	0	?	?	?	?	?	?

* PDSI, Palmer Drought Stress Index for Texas during July.

Table 2 Screwworm case incidence in relation to seasonal climatic conditions—analysis of deviance

Winter cases (W_n)		Sum of squares	d.f.	Mean square	F	R ²
Model		48.47	2	24.24	24.48	0.74
Residual		16.89	17	0.99		
Parameter	Estimate	s.e.	t	P		
a (intercept)	-16.24	6.843	3.35	<0.01		
b (autumn cases)	0.451	0.067	6.67	<0.001		
c (winter temperature)	0.342	0.101	3.38	<0.01		
Seasonal cases (S_n)		Sum of squares	d.f.	Mean square	F	R ²
Model		115.78	2	57.89	17.54	0.67
Residual		56.12	17	3.30		
Parameter	Estimate	s.e.	t	P		
d (intercept)	69.25	25.83	2.68	<0.02		
e (winter cases)	0.963	0.244	3.95	<0.001		
f (summer temperature)	-0.803	0.316	2.54	<0.02		

gests that the number of winter cases and the number of seasonal cases *actually increased with increasing rates of sterile male release*, which is ludicrous. The reason for this curious result lies in the fact that the release rates were positively correlated with the numbers of reported cases ($r^2 = 0.61$, $P < 0.01$) because fly production and hence release rates were increased in an attempt to control the burgeoning screwworm population. The release rates are therefore not included in the model.

Thus, despite the sterile male release programme, screwworm case incidence in Texas appears to have been determined largely by seasonal climatic conditions, especially temperature. The outbreaks occurred in response to favourable conditions, namely, warm winters and cool/wet summers. Conversely, cold winters and hot/dry summers, being unfavourable,

caused case incidence to decrease. This is illustrated in Fig. 1.

The actual coincidence between outbreaks on the one hand and favourable seasonal conditions on the other is shown graphically in Fig. 2.

Long-term trends

Having shown a clear relationship between seasonal climatic conditions and screwworm incidence in Texas, it is possible to examine the historical record for the southern United States with greater insight.

Long-term trends in winter temperatures for the relevant contiguous southern regions of the United States—South Atlantic, East South Central and West South Central—stretching westwards from Florida through Mississippi to Texas, as detailed by Diaz and Quayle²⁹, are illustrated in Fig. 3.

In respect of the South Atlantic Region, the appearance of the outbreaks in Florida in 1933 and their collapse in 1936 correspond nicely to the exceptionally warm winters of 1931–32, 32–33, and the very cold winter of 35–36 (Fig. 3a). Also the outbreaks between 1945 and 1957 occurred during a succession of warm winters and ended with a very cold winter in 1957–58, which, as noted earlier, fortunately coincided with the Florida eradication campaign. In fact in Florida, the 1957–58 winter was the coldest in nearly 100 years²⁶. Since 1958, most winters in Florida have been cold, some exceptionally so (for example, 1976–77 and 1977–78), except for a brief warm period in the early 1970s.

The East South Central temperature pattern (Fig. 3b) is similar but the trend towards colder winters during the past 25 years is even more pronounced. In

particular the cold winters of 1962–63, 63–64, 69–70 and 76–77 stand out.

Finally, the West South Central region (Fig. 3c), which includes Texas, confirms the cool trend, and, in conjunction with Fig. 2, illustrating more recent temperature trends in Texas, shows clearly that climatic conditions have been highly unfavourable for screwworm during the past 8 years, that is since the outbreaks collapsed in 1977. In Texas (see Fig. 2), the winters of 1977–78 and 1978–79 were the second and third coldest on record, whilst the 1980 summer was the second equal hottest on record (see Fig. 2). The 1983–84 winter was also exceptionally cold, killing citrus near San Antonio. The sudden downturn in screwworm case incidence in 1977 and the very low level since is therefore not surprising.

Predictions

Seasonal weather conditions, especially winter temperature but also summer moisture are often mentioned as important in relation to screwworm activity³⁰. Apparently, even in Mexico, hot dry summer weather is a major limiting factor¹⁷. However, Krafur¹⁸ concludes that climate was not important to the success of the eradication programme but his analytical method is inappropriate.

Past criticism of the programme has centred mostly on other factors such as strain fitness³¹, genetic isolation³², application rates and so on, particularly to account for the 1972–76 outbreaks in Texas.

The present analysis is much more damaging. At best it shows that, although the sterile releases may substantially suppress the screwworm population, they cannot prevent outbreaks developing in response to the occurrence of favourable seasonal conditions, at least not in Texas. At worst it suggests that the method may be ineffective and that eradication from the eastern States, namely Florida, occurred naturally because of a succession of very cold winters, combined perhaps with increased vigilance, more effective pesticides and changing land use.

Indeed it is quite possible that the screwworm, which is after all a tropical species, might become exceedingly scarce during periods of unfavourable climate in areas marginal to its normal distribution. It could exist at very low population levels in small isolated pockets provided by waterholes and rivers without being detected by man. Evidence for this exists in the Texas State Program Reports²⁷ which refer to an estimated ratio of 100:1 actual to reported cases during the 1976 summer season. Clearly zero reported cases does not necessarily mean eradication.

It is also possible that an exceptionally cold winter such as that occurring in Florida in 1957–58, or a succession of cold winters, could actually eradicate the

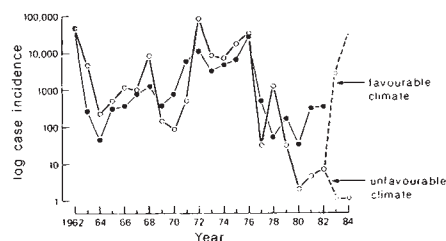


Fig. 4 Observed incidence (○) of screwworm in Texas during the 1962–82 eradication campaign compared with predicted incidence (●) based on actual winter and summer temperatures. Dashed lines represent predictions given favourable and unfavourable conditions.

species from areas isolated from the centre of the flies distribution. In this respect it is significant that the screwworm was not known in Florida before the exceptionally warm winter of 1931–32; that it appears to have reached there by natural progression eastward from Texas and Louisiana; and that winter temperatures in the South Atlantic Region appear to have been favourable for the fly only during the mid 1930s, 1950s and, to a lesser extent, the mid-1970s, in correspondence with the documented outbreaks. Before 1932 and after 1972 the winter climate seems to have been much less favourable for the fly in all the southern and eastern states (Fig. 3).

Admittedly eradication *per se* is virtually impossible to prove. But it is possible to verify the opposite view by making observations over sufficiently long periods of time in favourable potential habitats of the species concerned. In particular, in view of the current work in Mexico and the importance placed on the Tehuantepec barrier, it is imperative that strenuous efforts be made to detect the presence of screwworm in potentially favourable seasons and habitats throughout northern Mexico and southern Texas. Should these efforts fail, then eradication may more

safely be assumed, with the corollary that more emphasis be placed on restricting the movements of stock.

If eradication has not been achieved then my analysis leads to the prediction that the outbreaks will return, despite the Mexican programme. Even the additional weaponry known as SWASS (screwworm adult suppression system), an aerially distributed toxic-bait, is unlikely to prevent screwworm increase under favourable climatic conditions³³.

Figure 4 compares the observed incidence of screwworm cases in Texas over the last 20 years with those predicted by the model—starting with 49,000 cases in 1962 and using the winter and summer temperatures actually experienced in Texas (from Table 1). The model assumes that autumn cases (A_n) always represent one-third of the previous year's seasonal cases (S_n), an average determined from the actual case records listed in Table 1. The model shows also the expected increase or decrease over two seasons from the observed 1982 level (six cases) in response to either favourable or unfavourable seasonal conditions, based on the extremes observed during the 20-year period. Unfortunately there is no independent set of data on which to test the model, but if it is correct, we may expect screwworm outbreaks to re-occur in Texas in response to the return of favourable climatic conditions—namely, mild winters and cool, moist summers. I sincerely hope not, but my money is on the fly!

Since this manuscript was submitted there has been an outbreak of screwworm comprising "almost 150 reported infestations in northeastern Mexico (as near as 150 miles from the US border) during June and July 1985". □

J. L. Readshaw is a Senior Principal Research Scientist with the CSIRO, Division of Entomology, Canberra, ACT, Australia.

1. Knippling, E. F. in *Sterile Insect Technique and Radiation in Insect Control* 3–23 IAEA, Vienna, 1982.
2. Snow, J. W., Siebenaler, A. J. & Newell, F. G. *U.S. Dept. Agric. No. ARM-S-14*, 1–32 (1981).
3. Lindquist, A. W. *J. Econ. Entomol.* **48**, 467–469 (1955).
4. Knippling, E. F. *The Basic Principles of Insect Population Suppression and Management* (Agriculture Handbook No. 512, US Department of Agriculture, Washington, 1979).
5. Bushland, R. C. & Hopkins, D. E. *J. Econ. Entomol.* **44**, 725–731 ((1951); **46**, 648–656 (1953)).
6. Bushland, R. C. *Adv. vet. Sci.* **6**, 1–18 (1960).
7. Baumhover, A. H. *et al. J. Econ. Entomol.* **48**, 462–466 (1955).
8. Baumhover, A. H., Husman, C. N., Skipper, C. C. & New, W. D. *J. Econ. Entomol.* **52**, 1202–1206 (1959).
9. Baumhover, A. H. *J. Am. Med. Assoc.* **196**, 240–248 (1966).
10. Bushland, R. C. *Bull. Entomol. Soc. Am.* **21**, 23–26 (1975).
11. Whitten, C. J. in *Sterile Insect Technique and Radiation in Insect Control*, Ed. IAEA (Vienna, 1982), pp. 79–83.
12. Graham, O. H. & Hourigan, J. L. *J. Med. Entomol.* **13**, 629–657 (1977).
13. Williams, D. L., Gartman, S. C. & Hourigan, J. L. *World Anim. Rev.* **21**, 31–35 (1977).
14. Tannahill, F. H., Coppedge, J. R. & Snow, J. W. *J. Med. Entomol.* **17**, 265–267 (1980).
15. Rawlins, S. C., Alexander, F. C., Moe, V., Caesar, E., Moll, K. & Applewhite, L. *J. Econ. Entomol.* **76**, 1106–1111 (1983).
16. Krafur, E. S., Hightower, B. G., Vargas, M. J. *Med. Entomol.* **17**, 235–241 (1980).

17. Krafur, E. S. & Hightower, B. G. *J. med. Entomol.* **16**, 33–42 (1979).
18. Krafur, E. S. *Entomol. exp. appl.* **37**, 297–305 (1985).
19. Dove, E. W. & Parman, D. C. *J. Econ. Entomol.* **28**, 765–772 (1935).
20. Robinson, J. M. *J. Econ. Entomol.* **28**, 777–779 (1935).
21. Bissell, Theo. L. *Ga. Agric. exp. Sta. Bull.* **189**, 3–11 (1935).
22. Laake, E. W. *J. Econ. Entomol.* **28**, 648–649 (1935).
23. Dove, E. W. & Bishopp, F. C. *J. Econ. Entomol.* **29**, 1076–1085 (1936).
24. King, W. V. & Bradley, G. H. *J. Econ. Entomol.* **28**, 772–775 (1935).
25. Smith, A. L. & Skipper, C. C. *Fla. Entomol.* **35**, 10–13 (1951).
26. Karl, T. R., Metcalf, L. K., Nicodemus, M. L. & Quayle, R. G. *Statewide Average Climatic History. Texas 1888–1982. Florida 1891–1892* (Historical Climatology Series 6–1, National Climatic Data Center, Asheville, North Carolina, 1983).
27. Monthly program reports, Texas Agricultural Extension Service (1980).
28. Hightower, B. G. & Graham, O. H. in *Control of livestock insect pests by the Sterile Male Technique* 51–54 (IAEA, Vienna, 1968).
29. Diaz, H. F. & Quayle, R. G. *Mon. Weath. Rev.* **106**, 1393–1421 (1978).
30. Rahn, J. J. & Barger, G. L. *Agric. Meteorol.* **11**, 197–211 (1973).
31. Bush, G. L., Neck, R. W. & Kitto, G. B. *Science* **193**, 491–493 (1976).
32. Richardson, R. H., Ellison, J. R. & Averhoff, A. W. *Science* **215**, 361–370 (1982).
33. Coppedge, J. R. *et al. J. Econ. Entomol.* **71**, 579–584 (1978).

Latest Miocene benthic $\delta^{18}\text{O}$ changes, global ice volume, sea level and the 'Messinian salinity crisis'

David A. Hodell, Kristin M. Elmstrom & James P. Kennett

Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island 02882, USA

Oxygen isotope evidence indicates high but variable $\delta^{18}\text{O}$ values in benthic foraminiferal calcite during the latest Miocene and earliest Pliocene. These high values may represent increases in global ice volume and associated sea-level fall. The $\delta^{18}\text{O}$ record resembles glacial/interglacial cycles, but with only one-third the amplitude of the late Pleistocene signal. This variability may reflect instability in the Antarctic ice sheet, and palaeomagnetic correlation points to an isotopic event coinciding with the isolation and desiccation of the Mediterranean basin during the latest Messinian.

DURING the Messinian Age of the late Miocene, the Mediterranean Sea was isolated from the world ocean, and thick and extensive evaporites were deposited in pre-existing basins of the proto-Mediterranean Sea (the 'Messinian salinity crisis'¹). The closure of the Mediterranean was controlled by a combination of tectonic compression in the western Mediterranean and a global sea level fall during the latest Miocene¹⁻³. In 1977, Adams *et al.*³ stated that "the final severing (between the Mediterranean and Atlantic) may have resulted from glacial-eustatic lowering of the global ocean. Further stratigraphic resolution of late Miocene sea level and ice-volume changes is sought to verify the eustatic-fall hypothesis." We address this question here by examining high-resolution benthic oxygen isotopic data from six sites in the Atlantic and Pacific oceans to determine possible relationships between changes in the benthic $\delta^{18}\text{O}$ record and deposition of the Messinian evaporites.

The eustatic fall during the latest Miocene has often been attributed to an increase in the volume of ice on Antarctica, but different sets of oxygen isotope data have been found to be contradictory. For example, Shackleton and Kennett⁴ proposed an expansion of the Antarctic ice sheet to 1.5–1.8 times its present size, based on a 0.5‰ increase in oxygen isotopic ratios during the latest Miocene Kapitean Stage at DSDP Site 284, South-West Pacific. The increase at Site 284 is somewhat tenuous, based upon two points only. Furthermore, isotopic analyses of other sections (DSDP Sites 158, 310 and 281) showed no evidence of a single large increase in latest Miocene $\delta^{18}\text{O}$ values⁵⁻⁷. This contradiction led Ciesielski *et al.*⁸ to conclude that "further oxygen isotopic studies of late Miocene deep-sea sediments from the southern high latitudes are obviously needed to clarify the true nature of oxygen isotopic change during the latest Miocene."

We have reanalysed Site 284 at a higher sampling frequency to support or reject the $\delta^{18}\text{O}$ increase reported by Shackleton and Kennett⁴. We have also analysed DSDP Site 516A⁹ and three piston cores from *Chain* Cruise 115 from the Rio Grande Rise in the western South Atlantic. Shackleton and Cita's data from Site 397 in the North Atlantic were included because this site has been correlated to the Messinian salinity crisis^{10,11}. From Leg 90 in the South-West Pacific, we analysed Sites 588 and 590¹², which offer excellent stratigraphic resolution because of high sedimentation rates in the latest Miocene-early Pliocene.

The placement of the Miocene/Pliocene (M/P) boundary is important to this study because its definition is intimately tied to the re-establishment of open marine conditions in the Mediterranean following the Messinian salinity crisis¹³. The boundary stratotype is at the base of the Zanclean Stage at Capo Rosello, Sicily, at the contact between the underlying pyritic

clays and sands of the Arenazzolo unit and overlying Trubi marls. In the latest revision of the Neogene timescale, Berggren *et al.*¹⁴ suggested that the M/P boundary falls just above the Chron 5/Gilbert boundary at 5.3 Myr. The M/P boundary in Site 588 was placed at 107 m, based on the first appearance datum (FAD) of *Globorotalia tumida* and the last appearance datum (LAD) of *Globorotalia conomiozea*¹⁵. The boundary in Site 588 falls in the lower part of the Gilbert at about 5.0 Myr, or 300,000 yr above the Chron 5/Gilbert boundary. Preliminary cross-correlation of ⁸⁷Sr/⁸⁶Sr ratios from the base of the Zanclean with magnetostratigraphy from DSDP Site 519 suggests that the base of the Trubi at Capo Rosello is several hundreds of thousands of years above the Chron 5/Gilbert boundary¹⁶.

Presentation of data

The benthic oxygen isotope record from Site 588 is shown in Fig. 1. Between 80 and 160 m, we sampled every 70 cm (a sampling interval of 20–25 kyr). The time control is excellent in Site 588 because of palaeomagnetic reversal stratigraphy¹⁷; the interval of high sampling resolution extends from 6.4 to 3.9 Myr. Oxygen isotopic ratios during the latest Miocene-early Pliocene are clearly marked by high-frequency variation (Fig. 1). The amplitude of the $\delta^{18}\text{O}$ signal is about 0.5‰. In the Quaternary, the maximum difference between glacial and interglacial $\delta^{18}\text{O}$ values is 1.6–1.8‰; the amplitude of the latest Miocene-early Pliocene fluctuations is thus one-third of the late Pleistocene signal.

In Fig. 1, 3- and 5-point running averages of the raw data are presented, to show lower-frequency components of $\delta^{18}\text{O}$ variation. Oxygen isotopic fluctuations during the latest Miocene-early Pliocene resemble typical glacial-interglacial cycles of the Quaternary, except in their smaller amplitude. The period of these cycles, when the data are plotted against age derived from magnetostratigraphy, is ~400,000 yr. Cyclicity of this period has been reported to dominate late Miocene carbonate records from the Pacific¹⁸, and may reflect the natural period of 'glacial-interglacial' oscillation in Antarctic ice volume during the late Miocene.

In the following description of the data, we use the terms 'glacial' and 'interglacial' to refer to episodes of net increase and decrease in $\delta^{18}\text{O}$, respectively, although we acknowledge that 'cool' and 'warm' are equally applicable because we cannot separate the temperature signal from the ice volume signal in the $\delta^{18}\text{O}$ record. The intensity of glacial and interglacial cycles appears to increase progressively through the latest Miocene and early Pliocene. The first prominent glacial interval (increased $\delta^{18}\text{O}$ values) occurs in the younger normal event of Chron 5 between 5.5 and 5.2 Myr. This is followed by a short-

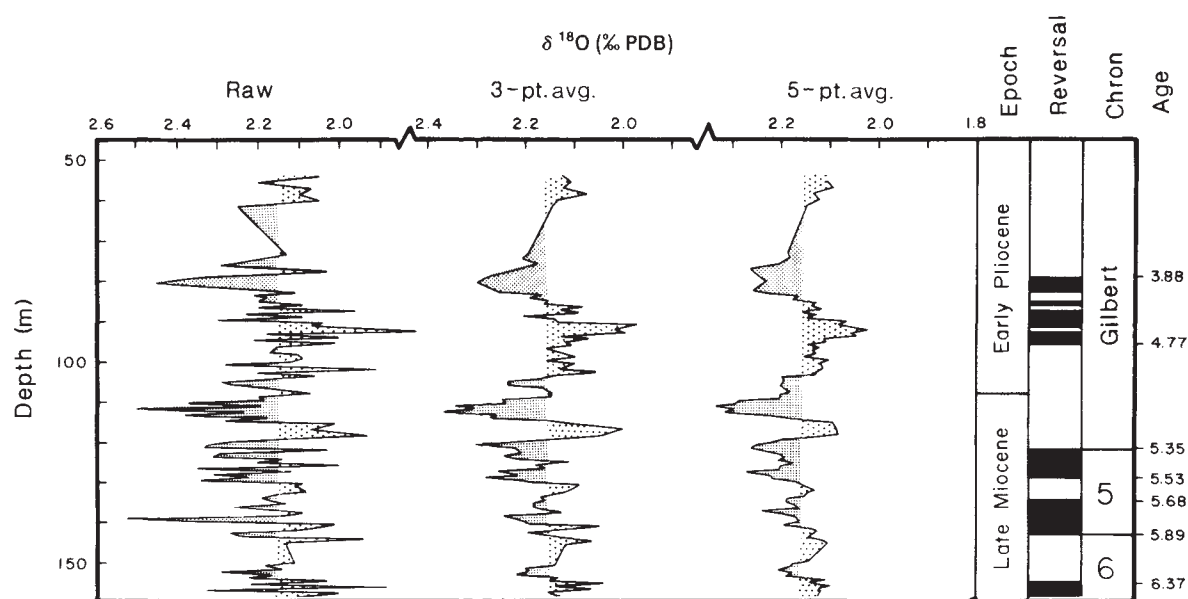


Fig. 1 Oxygen isotope results from benthic foraminifers *Planulina wuellerstorfi* and/or *Cibicides kullenbergi* from Site 588, DSDP Leg 90, South-West Pacific. The sampling interval is 20–25 kyr between 80 and 160 m, or between 6.4 and 3.9 Myr if ages¹⁴ are assigned to the magnetobiostratigraphy of Barton and Bloemendal¹⁷. The raw $\delta^{18}\text{O}$ data clearly show that the latest Miocene–early Pliocene was characterized by high-frequency variation in $\delta^{18}\text{O}$. The amplitude of variation is $\sim 0.5\%$ (one-third of the late Pleistocene signal), and the wavelength is $\sim 400,000$ yr. The 3- and 5-point running averages of the data show the general trends in $\delta^{18}\text{O}$, and resemble glacial–interglacial cycles.

term interglacial just above the Chron 5/Gilbert boundary between 5.2 and 5.1 Myr. Oxygen isotopic ratios reach a maximum in the lower Gilbert between 5.1 and 4.8 Myr, reflecting maximum intensity of glaciation or cooling. An interval of lower $\delta^{18}\text{O}$ values is apparent in the early Pliocene between 4.8 and 4.1 Myr, marking a strong interglacial or warming. The sampling interval decreases above 80 m, but the record indicates that glacial and interglacial events occurred throughout the remaining Pliocene.

Figure 2 is a composite of oxygen isotopic data from six sites (Table 1). The chronology is based on the palaeomagnetic reversal stratigraphy of Sites 588 and 397^{17,19}. The other curves were aligned by matching oxygen isotope records between sites. The biostratigraphic position of the M/P boundary is shown for each site, and generally supports the isotopic correlation. The position of the boundary is uncertain by 0.5 Myr (at ~ 5.5 –5.0 Myr), which is reasonable considering the inadequacies of biostratigraphic recognition of the boundary. All sites show an interval of relatively high $\delta^{18}\text{O}$ values occurring near the M/P boundary. The differences in the character of the $\delta^{18}\text{O}$ records between sites results from different sampling resolution and sedimentation rates.

The benthic $\delta^{18}\text{O}$ record at Site 397 is remarkably similar to that at Site 588. Shackleton and Cita¹⁰ reported high-frequency $\delta^{18}\text{O}$ variation occurring just above and below the position of the Chron 5/Gilbert boundary. Oxygen isotopic ratios increase in late Chron 5, decrease near the Chron 5/Gilbert boundary, increase again in the early Gilbert, and then decrease in the early Pliocene. Maximum $\delta^{18}\text{O}$ values were calculated to be 0.3‰ more positive than Holocene values, and the amplitude of the $\delta^{18}\text{O}$ variation was reported to be about one-third of the late Pleistocene signal¹⁰.

Site 590 shows high $\delta^{18}\text{O}$ values in the younger part of Chron 6, and in the very latest Miocene in late Chron 5 and early Gilbert, just before the biostratigraphic position of the M/P boundary. At Site 284, the $\delta^{18}\text{O}$ increase reported by Shackleton and Kennett⁴ is confirmed with more data; however, the increase is no longer single-peaked, but quite broad, spanning the M/P boundary. Following the interval of high $\delta^{18}\text{O}$

values, oxygen isotopic ratios show a distinct decrease in the early Pliocene. At Site 516A, oxygen isotopic ratios are similar throughout much of the late Miocene and early Pliocene, except for anomalously high $\delta^{18}\text{O}$ values above and below the biostratigraphic placement of the M/P boundary. These extreme values were reproduced by analysing replicate samples, which gave indistinguishable results within analytical error. Site 516A was sampled every 50,000 yr, and the low resolution of the sampling at this site is probably responsible for the compressed nature of the $\delta^{18}\text{O}$ increase. Oxygen isotopic ratios at Site 516A show a prominent decrease near the top of the record in the early Pliocene. At Chain site (CH) 115, we sampled three piston cores at 10–20-cm intervals to provide higher resolution over the M/P boundary in the South Atlantic. The oxygen isotope record of the individual piston cores (Chain 64, 67 and 86PC) shows similar trends; the data from the three cores were stacked to produce the composite record shown in Fig. 2. The CH 115 data clearly show a distinct interval of higher $\delta^{18}\text{O}$ values associated with the M/P boundary, followed by a trend toward decreasing $\delta^{18}\text{O}$ values in the early Pliocene.

All sites show an increase in $\delta^{18}\text{O}$ values in the latest Miocene, as originally observed by Shackleton and Kennett⁴ at DSDP Site 284. But whereas the Shackleton and Kennett data⁴ show a single point-increase in $\delta^{18}\text{O}$, the higher-resolution records demonstrate that the latest Miocene was marked by high-frequency $\delta^{18}\text{O}$ variation consisting of several ‘cool-glacial’ and

Table 1 Sample localities

Site	Latitude	Longitude	Water depth
Chain 115	30°00' S	35°00' W	2,100 m
DSDP 284*	40°31' S	167°41' E	1,066 m
397†	20°51' S	15°11' W	2,900 m
516A‡	30°17' S	35°17' W	1,313 m
588	26°06' S	161°13' E	1,533 m
590§	31°10' S	163°22' E	1,299 m

* See also ref. 4. † See ref. 10. ‡ See also ref. 9. § See also ref. 12.

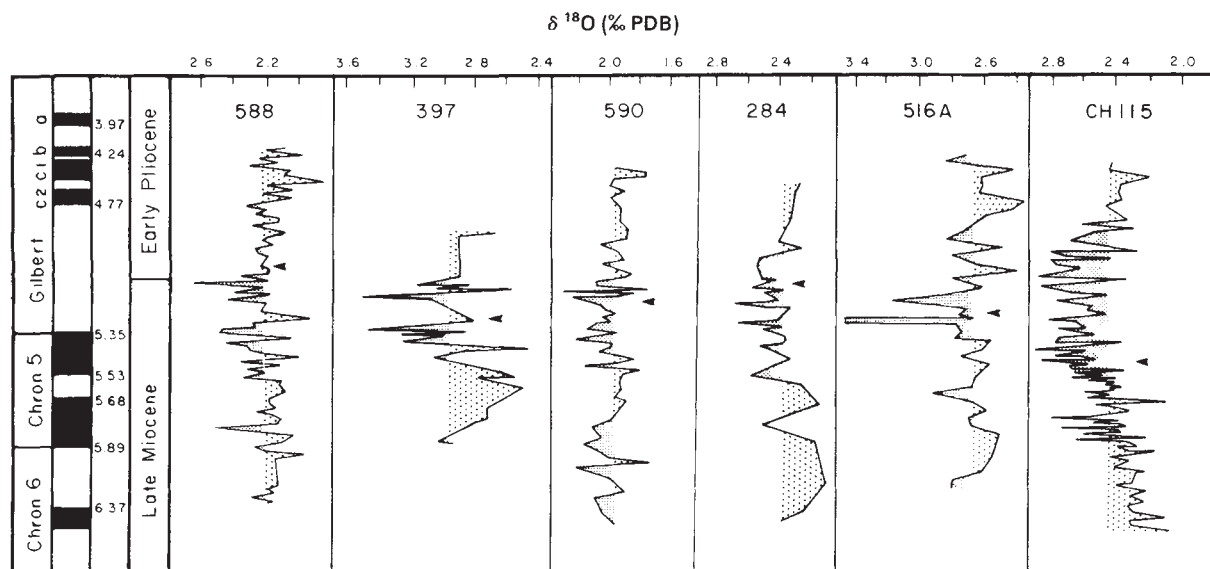


Fig. 2 Oxygen isotopic results from Sites 588 (South-West Pacific), 397 (North Atlantic), 590, 284 (South-West Pacific), 516A, and CH115 (South-West Atlantic) over the M/P boundary. Oxygen isotopic analyses were generated on the benthic foraminifers *Planulina wuellerstorfi* and/or *Cibicidoides kullenbergi* at Sites 588, 590¹², 516A⁹ and CH 115; several species were run at Site 397 and adjusted to *Uvigerina peregrina*¹⁰; *Uvigerina* spp. were analysed in Site 284⁴. The palaeomagnetic stratigraphy for Sites 588 and 397 is after Barton and Bloemendal¹⁷ and Hamilton¹⁹, respectively; ages assigned to magnetic boundaries are after Berggren *et al.*¹⁴. The M/P boundary was placed in the lower Gilbert at 107 m at Site 588, based on the FAD of *Globorotalia tumida* and LAD of *Globorotalia conomiozea*¹⁵. The biostratigraphic placement of the M/P boundary is indicated by an arrow. All sites show a general increase in $\delta^{18}\text{O}$ values in the latest Miocene as originally reported by Shackleton and Kennett⁴ at Site 284, but the high-resolution record of Site 588 demonstrates that the latest Miocene was also marked by high-frequency $\delta^{18}\text{O}$ changes consisting of several 'cool-glacial' and 'warm-interglacial' episodes.

'warm-interglacial' intervals. The data from Site 588 suggest that maximum $\delta^{18}\text{O}$ values occur during late Chron 5 and early Gilbert. The time interval dominated by high $\delta^{18}\text{O}$ values is ~500,000 yr, brief enough to be easily missed by inadequate sampling, poor core recovery or removal by erosion or dissolution. These factors have undoubtedly contributed to the reported absence of an increase in late Miocene $\delta^{18}\text{O}$ values in other studies⁵⁻⁷.

Ice volume changes

The high-frequency $\delta^{18}\text{O}$ variations in the latest Miocene and early Pliocene suggest that ice volume and/or temperature changes occurred throughout this interval. With benthic oxygen isotopic data alone, we cannot deconvolve the isotopic signal into its temperature and ice volume components; however, the fact that we observe similar benthic $\delta^{18}\text{O}$ patterns in six different sites, located at different depths and in different ocean basins, suggests that these changes were at least partially controlled by compositional changes in the $\delta^{18}\text{O}$ of sea water due to fluctuations in global ice volume.

Many authors have suggested an expansion of ice on Antarctica during the latest Miocene, including the development of the West Antarctic ice sheet^{8,20-24}. Mercer suggested that ice rafting would abruptly increase its range as ice shelves and floating ice tongues first formed in the Antarctic, because tabular icebergs carry several orders of magnitude more load than do temperate outlet glaciers²⁵. During the latest Miocene, the Antarctic Convergence moved northward by 300 km and the distribution of ice-rafted detritus abruptly expanded equatorward^{22,23,26}. For example, ice-rafted detritus became conspicuous for the first time on the Falkland Plateau at 50°S in the Atlantic⁸. Drewry suggested that conditions suitable for the formation of the marine-based West Antarctic ice sheet did not occur until the late Miocene (4-5 Myr)²¹. Although the age of the Ross Sea disconformity is controversial, several authors suggest that it was formed during the late Miocene to early

Pliocene and indicates the presence of grounded ice in West Antarctica^{8,21}.

Ciesielski *et al.* concluded that the sedimentary record of the Maurice Ewing Bank precludes the existence of a West Antarctic ice sheet or extensive ice shelves along the Antarctic margin before the late Miocene⁸. Widespread erosion of the Maurice Ewing Bank in the latest Miocene is inferred to have been caused by the first production of 'true' Antarctic Bottom Water (AABW)⁸, formed by the cooling of surface waters beneath the vast ice shelf that would have extended over much of West Antarctica in the latest Miocene, before the central part of the West Antarctic ice sheet had been grounded.

The mid-latitude history of Southern Hemisphere glaciation supports the general oxygen isotopic trends presented here. The earliest known tills from Argentine Patagonia are latest Miocene in age, and reflect glacial expansion beyond the Andean Mountain front²⁷. This glacial evidence provides a minimum age for the initial growth of the West Antarctic ice sheet to the south of South America. The latest Miocene Patagonian glaciation is followed by an interglacial interval during the early Pliocene, and a renewed glacial advance at about 3.6 Myr.

The amplitude and frequency of $\delta^{18}\text{O}$ changes, coupled with ancillary sedimentological and glaciological evidence, suggest that substantial changes occurred in global ice volume during the latest Miocene and early Pliocene. Three possible loci of ice growth and decay exist: East Antarctica, West Antarctica and the Northern Hemisphere. Part of the $\delta^{18}\text{O}$ signal could reflect ice-volume instability resulting from periodic grounding and ungrounding of the West Antarctic ice sheet during the early stages of its formation. Even today the West Antarctic ice sheet is inherently unstable and highly susceptible to small changes in high-latitude summer temperatures²⁸. Calculations show that formation of the West Antarctic ice sheet would not dramatically alter the oxygen isotopic composition of sea water because of its small volume ($1.5-1.8 \times 10^6 \text{ km}^3$) and relatively high $\delta^{18}\text{O}$ (-20 to -30‰)⁸.

The Messinian salinity crisis

We suggest a strong correspondence between the trends in the oxygen isotopic data and the deposition of the Messinian evaporites. Correlations between $\delta^{18}\text{O}$ and evaporite deposition have also been discussed previously^{10,29}. The Messinian evaporites were deposited during two discrete stages in late Chron 5 and earliest Gilbert^{15,30,31}. The lower evaporite ('Main Salt') unit contains thick halite deposits associated with potassium salts. The great thickness (>1 km) of salt implies a continuous supply of marine waters, and communication between the Mediterranean and the global ocean throughout much of the salinity crisis. Within the Main Salt unit, sedimentological evidence indicates many cycles of marine flooding and evaporative drawdown of water, followed by subaerial erosion^{30,31}.

Towards the end of deposition of the Main Salt unit, the supply of brine from the Atlantic was probably cut off because of near-complete isolation of the Mediterranean³⁰. This desiccation interval is represented by a widespread unconformity separating the lower and upper evaporite units, and included erosion and recycling of primary salts from the periphery of the basins. This erosional event was followed by an intra-Messinian transgression, when the Mediterranean may have been refilled to the brim³⁰. Next, the upper evaporite unit was deposited, consisting of cyclic deposition of marls, stromatolitic carbonates, gypsum, anhydrites and salts. These cycles represent repetition of marine inundation, isolation and evaporative drawdown. Evaporite deposition ceased in the early Pliocene, when tectonic breakage of the dam at Gibraltar and a marine transgression refilled the Mediterranean, resulting in the restoration of deep-water pelagic sedimentation.

The similar character, timing and duration of trends in the oxygen isotopic data and the Messinian salinity crisis imply a causal relationship. The final isolation of the Mediterranean was controlled by long-term tectonic uplift of the western Mediterranean as well as rapid sea-level lowering during the latest Miocene³. The relatively high $\delta^{18}\text{O}$ values in the younger normal event of Chron 5 record an ice-volume increase and eustatic sea-level fall that contributed to the isolation of the Mediterranean. The final connection between the Atlantic and the Mediterranean is believed to have been through the Betic Straits in southern Spain and/or Rif Straits in northern Morocco³. Berggren and Haq³² proposed an abrupt drop in sea level of 50–70 m at 5.5 Myr from benthic foraminiferal assemblage changes in the Andalusian stratotype at Carmona, Spain. The isolation of the Betic Straits at 5.5 Myr coincides with the increase in $\delta^{18}\text{O}$ values reported here at the base of the younger normal event of Chron 5 (5.6–5.5 Myr).

The lower and upper evaporite units are often separated by an unconformity marking near-complete desiccation of the basin. Marine marls overlie the unconformity in some sections,

and indicate an interval of marine transgression that partly or totally refilled the Mediterranean³⁰. This intra-Messinian transgression may be represented by the relatively low $\delta^{18}\text{O}$ values that occur near the Chron 5/Gilbert boundary in several sites (588, 397, 516A). Increased sampling resolution and examination of the palaeomagnetic stratigraphy of other high-quality sections are needed to confirm this suggested correlation.

Maximum $\delta^{18}\text{O}$ values occur in the Lower Gilbert (5.1–4.8 Myr), which we correlate with the latest Miocene or uppermost Messinian. These high, variable $\delta^{18}\text{O}$ values appear to coincide with the cyclic deposition of the upper evaporite unit. The high-frequency variation, superimposed on a lower-frequency component (400,000-yr period), may represent sea-level oscillations that controlled the flux of marine waters across the sills separating the Atlantic and the Mediterranean during the latest Miocene. Periodic inundation of marine waters into a largely desiccated Mediterranean resulted in the cyclic deposition of the upper evaporite unit. During the early Pliocene between ~4.8 and 4.1 Myr, all sites show a decrease in $\delta^{18}\text{O}$ reflecting a strong interglacial or warming. The early Pliocene has been characterized as a time of global warmth^{4,33}, and is transgressive in many shallow marine sequences throughout the world^{32,34}. The decrease in $\delta^{18}\text{O}$ in the early Pliocene coincides with termination of evaporite deposition and restoration of open-marine conditions in the Mediterranean.

As other authors have suggested^{1,2,3,11,29,30,35}, we speculate that the isolation and desiccation of the Mediterranean during the latest Miocene was controlled, in part, by glacio-eustatic changes as recorded by benthic oxygen isotopic ratios. Latest Miocene-early Pliocene oxygen isotope records are marked by high-frequency changes that resemble Quaternary glacial-interglacial cycles. The amplitude is about one-third of the late Pleistocene signal, and the wavelength is ~400,000 yr. Increased $\delta^{18}\text{O}$ values at the base of the younger normal event of Chron 5 coincide with a drop in sea level at 5.5 Myr, recorded in the Carmona Dos-Hermanos section in southern Spain³². This eustatic lowering is believed to have severed the final connection between the Atlantic and the Mediterranean, and permitted the onset of evaporite deposition. In some sites, a negative excursion in $\delta^{18}\text{O}$ occurs near the Chron 5/Gilbert boundary between 5.2 and 5.1 Myr, and may correspond with an intra-Messinian transgression that temporarily refilled the Mediterranean. Maximum $\delta^{18}\text{O}$ values occur in the Lower Gilbert between 5.1 and 4.8 Myr, and correspond with the deposition of the upper evaporite unit. The evaporites show evidence of distinct cyclicity, which may have been controlled by sea-level changes related to the waxing and waning of latest Miocene ice sheets^{2,11,29,30,35}, as suggested by high-frequency $\delta^{18}\text{O}$ changes. A rapid decrease in $\delta^{18}\text{O}$ is evident at all sites during the early Pliocene at about 5.0 Myr, marking a glacial retreat and marine transgression that coincided with the termination of the salinity crisis and restoration of open-marine conditions in the Mediterranean.

Received 25 July 1985; accepted 11 February 1986.

- Hsu, K. J., Cita, M. B. & Ryan, W. B. F. *Init. Rep. DSDP* 13, 1203–1231 (1973).
- Ryan, W. B. F. in *Messinian Events in the Mediterranean* (ed. Drooger, C. W.) 26–38 (North-Holland, Amsterdam, 1973).
- Adams, C. G. *et al. Nature* 269, 383–386 (1977).
- Shackleton, N. J. & Kennett, J. P. *Init. Rep. DSDP* 29, 801–807 (1975).
- Keigwin, L. D. Jr *Earth planet. Sci. Lett.* 45, 361–382 (1979).
- Loutit, T. S. & Kennett, J. P. *Science* 204, 1196–1199 (1979).
- Loutit, T. S. *Mar. Micropaleont.* 16, 1–27 (1981).
- Ciesielski, P. F., Ledbetter, M. T. & Elwood, B. B. *Mar. Geol.* 46, 1–51 (1982).
- Leonard, K. A., Williams, D. F. & Thunell, R. C. *Init. Rep. DSDP* 72, (1983).
- Shackleton, N. J. & Cita, M. B. *Init. Rep. DSDP* 47, 433–445 (1979).
- Cita, M. B. & Ryan, W. B. F. *Init. Rep. DSDP* 47, 447–459 (1979).
- Elmstrom, K. M. & Kennett, J. P. *Init. Rep. DSDP* 90, 1361–1381 (1986).
- Cita, M. B. in *Late Neogene Epoch Boundaries* (eds Tsunemasa, S. & Burckle, L. H.) 1–30 (Micropaleontology Press, New York, 1975).
- Berggren, W. A., Kent, D. V. & Van Couvering, J. A. in *Geochronology and the Geological Record* (ed. Snelling, N. J.) (Geological Society of London, in the press).
- Jenkins, D. G. & Srinivasan, M. S. *Init. Rep. DSDP* 90, 795–834 (1986).
- McKenzie, J. A., Mueller, P. & Mueller, D. *Geol. Soc. Am. Abstr. Progr.* 17, 659 (1985).
- Barton, C. E. & Bloemendal, J. *Init. Rep. DSDP* 90, 1273–1276 (1986).

- Moore, T. C. Jr Pisias, N. G. & Dunn, D. A. *Mar. Geol.* 46, 217–233 (1982).
- Hamilton, N. *Init. Rep. DSDP* 47, 463–477 (1979).
- Kennett, J. P. N. Z. *J. Geol. Geophys.* 10, 1051–1063 (1967).
- Drewry, D. J. in *Antarctic Glacial History and World Palaeoenvironments* (ed. Van Zinderen Bakker, E. M.) 25–32 (Balkema, Rotterdam, 1978).
- Hayes, D. E. & Frakes, L. A. *Init. Rep. DSDP* 28, 919–942 (1975).
- Plafker, G., Bartsch-Winkler, S. & Overshine, A. T. *Init. Rep. DSDP* 36, 857–867 (1977).
- Mercer, J. H. A. *Rev. Earth planet. Sci.* 11, 99–132 (1983).
- Mercer, J. H. in *Palaeoecology of Africa* Vol. 8 (ed. Van Zinderen Bakker, E. M.) 885–114 (Balkema, Rotterdam, 1973).
- Kemp, E. M., Frakes, L. A. & Hayes, D. E. *Init. Rep. DSDP* 28, 909–917 (1975).
- Mercer, J. H. & Sutter, J. F. *Paleogeogr. Paleoclimatol. Paleocool.* 38, 185–206 (1982).
- Mercer, J. H. *Nature* 271, 321–325 (1978).
- McKenzie, J. A. & Oberhänsli, H. in *South Atlantic Paleooceanography* (eds Hsu, K. J. & Weissert, H. J.) 99–123 (Cambridge University Press, 1985).
- Hsu, K. J. *et al. Nature* 267, 399–403 (1977).
- Hsu, K. J. *et al. Init. Rep. DSDP* 42, 1053–1077 (1978).
- Berggren, W. A. & Haq, B. U. *Paleogeogr. Paleoclimatol. Paleocool.* 20, 67–129 (1976).
- Kennett, J. P. *et al. Am. J. Sci.* 279, 52–69 (1979).
- Kennett, J. P. & Watkins, N. D. *Bull. geol. Soc. Am.* 85, 1385–1398 (1974).
- Cita, M. B. & Ryan, W. B. F. *Riv. Ital. Paleont. Stratigr.* 84, 1051–1082 (1978).

A new acute transforming feline retrovirus and relationship of its oncogene *v-kit* with the protein kinase gene family

Peter Besmer^{*}, John E. Murphy^{*§}, Patricia C. George^{*§}, Feihua Qiu^{*}, Peter J. Bergold^{*}, Lynn Lederman^{†§}, Harry W. Snyder Jr^{†§}, David Brodeur[‡], Evelyn E. Zuckerman[‡] & William D. Hardy[‡]

Laboratories of ^{*} Molecular Oncology, [†] Viral Oncology and [‡] Veterinary Oncology, Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center and Cornell University Graduate School of Medical Sciences, New York, New York 10021, USA

A new acute transforming feline retrovirus, the Hardy-Zuckerman 4 feline sarcoma virus (HZ4-FeSV), has been isolated from a feline fibrosarcoma. The viral genome of HZ4-FeSV contains a new oncogene designated v-kit, has the structure 5' Δgag-kit-Δpol-Δenv 3' and specifies a gag-kit polyprotein of relative molecular mass 80,000. The predicted kit amino-acid sequence displays partial homology with tyrosine-specific protein kinase oncogenes. HZ4-FeSV appears to have been generated by transduction of feline c-kit sequences with feline leukaemia virus.

VIRAL and non-viral oncogenes can be classified into four distinct groups according to their functional properties—protein kinases, GTPases, nuclear proteins and growth factors, the first of these groups being by far the largest. The functions of the normal cellular counterparts (*c-oncs*) are known for the oncogenes *sis*, *erb-B* and *fms*; the *v-sis* gene of the simian sarcoma virus and the Parodi-Irgens feline sarcoma virus (PI-FeSV) is the homologue of platelet-derived growth factor¹⁻³, the *erb-B* protein of avian erythroblastosis virus (AEV) is homologous with the epidermal growth factor receptor⁴, and the *c-fms* protein is related to the receptor for the macrophage colony-stimulating factor CSF-1 (ref. 5). The current notion is therefore that *c-oncs* are components of signal transduction pathways. Many of the known oncogenes were discovered through their association with retroviruses. Oncogenes of acute transforming retroviruses (*v-oncs*) are acquired by transduction of *c-onc* sequences by retroviruses and have been isolated in several vertebrate species from neoplasms of retrovirus-infected animals. Some *v-oncs* have been found in multiple virus isolates of the same species while others have re-emerged in viruses isolated from different species, indicating that the pool of *c-oncs* which can be transduced by retroviruses is limited.

Feline leukaemia virus (FeLV) is transmitted horizontally between domestic cats and is associated with a wide spectrum of neoplastic and blastopenic diseases. Like other retroviruses, FeLV can transduce *c-onc* sequences^{6,7}. The rare naturally occurring FeLV-associated feline multicentric fibrosarcomas have been a rich source of acute transforming retroviruses. Nine FeSV strains have been isolated from feline fibrosarcomas: Snyder-Theilen(ST), Gardner-Arnstein (GA), Susan McDonough (SM), Gardner-Rasheed (GR), PI, Theilen-Pederson (TP1) and Hardy-Zuckerman (HZ1 and HZ2)^{2,7-15}. Five different oncogenes have been identified in these viruses—*v-fes*, *v-fms*, *v-fgr*, *v-abl* and *v-sis*—indicating that there is no particular specificity for the transduction of *c-oncs* by FeLV^{2,15-18}. To investigate further the repertoire of proto-oncogenes that can be transduced by retroviruses, we have isolated and characterized another FeSV strain, the Hardy-Zuckerman 4 feline

sarcoma virus (HZ4-FeSV). This virus contains a unique oncogene, which we have designated *v-kit*, and the predicted *kit* amino-acid sequence displays partial homology with protein kinase oncogenes. We also describe the identification of FeLV *gag* sequences that are preferentially involved in the generation of acute transforming feline retroviruses.

Isolation of HZ4-FeSV

A 7-yr-old FeLV-infected male domestic cat (SKI 3637) with multiple fibrosarcomas was obtained from a pet household in St Louis, Missouri. The second digit of the right hind leg had been removed because of the presence of a solitary fibrosarcoma. A year after the initial surgery six fibrosarcomas recurred on the right hock and millary metastases were present in the lungs. The FeLV isolated from the serum and from tumour tissue of cat 3637 was determined as belonging to subgroups A and B (ref. 19). Infection of feline embryo fibroblasts (FEA) and CCL64 mink cells with 0.8 μm filtrate obtained from a homogenate of one of the primary tumours produced distinct foci of transformed cells. Mink HZ4-FeSV virus non-producer cells were obtained from which transforming virus could be rescued by superinfection with amphotropic murine leukaemia virus (MuLV). This result indicated the replication-defective nature of the HZ4-FeSV. HZ4-FeSV non-producer mink cells display characteristic parameters of transformation, that is, they grow in soft agar and they induce fibrosarcomas in nude mice.

Genetic structure of HZ4-FeSV

The structure of the integrated HZ4-FeSV provirus in the DNA of HZ4-FeSV-infected mink cells was first investigated by Southern blot analysis. A 9.6-kilobase (kb) *Hind*III restriction fragment, presumed to contain the entire HZ4-FeSV provirus, was identified using a FeLV hybridization probe (data not shown), and cloned using the λ vector Charon 30 (Fig. 1 legend). Figure 1A shows a restriction map of the integrated HZ4-FeSV provirus. Restriction fragments containing FeLV-related sequences were identified by hybridization of Southern blots with an FeLV *rep* probe and the genetic structure of the HZ4-FeSV provirus was confirmed by hybridization with probes specific for *gag* and *env* (data not shown). A *Sac*/*Sal* fragment of 0.75 kb (2.75–3.5 kb) was found to lack homology with FeLV sequences, indicating that this fragment contains the HZ4-FeSV *v-onc* sequences. We have designated the HZ4-FeSV-specific sequences *v-kit*. In agreement with the genetic structure of the HZ4-FeSV, a 4.5-kb genome RNA was detected in HZ4-FeSV-

§ Present addresses: Columbia University College of Physicians and Surgeons, New York, New York 10032, USA (J.E.M.); Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545, USA (L.L.); Pacific Northwest Research Foundation, Seattle, Washington 98104, USA (H.W.S.); Cornell University Medical School, New York, New York, 10021, USA (P.C.G.); Duke University Medical School, Durham, North Carolina 27710, USA (D.B.).

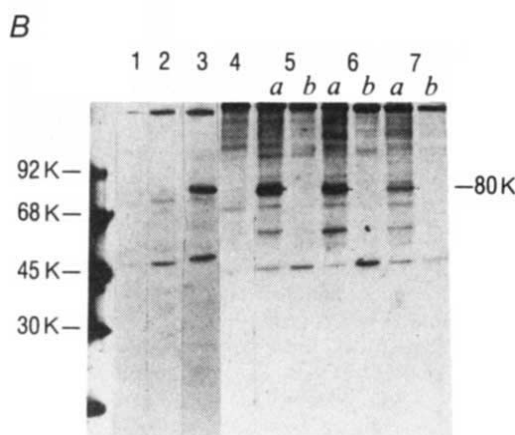
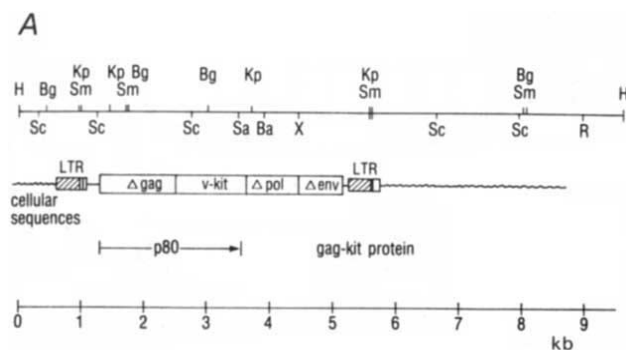


Fig. 2 Nucleotide sequence of the HZ4-FeSV *gag-kit* gene. The complete nucleotide sequence of the *gag-kit* gene sequence was determined by the dideoxynucleotide chain termination method of Sanger following subcloning of fragments into the M13 vectors mp18 and mp19 (refs 22, 49). The predicted amino-acid sequence of the only long open reading frame is shown above using single-letter amino-acid notations. The numbers at the end of each line refer to nucleotides with position +1 defining the beginning of the *gag* gene. The nucleotides determining the 74-amino-acid *gag* leader have been assigned negative numbers as proposed by Hampe *et al.*¹⁷. Junctions between the *gag* proteins p15, p12 and p30 are indicated as well as the 5' and 3' boundaries of the *v-kit* sequences. The *v-kit* nucleotide sequence was determined on both strands and all restriction sites were crossed; the *gag* nucleotide sequence, on the other hand, was determined at least on one strand. The 5' recombination site between *gag* and *kit* sequences was deduced by comparison of the *gag-kit* sequence with that of the known FeLV *gag* sequence^{17,23}. To determine the 3' recombination site between *v-kit* and FeLV *pol*, the *gag-kit* sequence was compared with the sequence of the GA-FeLV *pol* gene (*Sma/Kpn* fragment, 4.9–5.1 kb of the GA-FeLV map). The nucleotide sequence of the GA-FeLV *Sma/Kpn* *pol* fragment was determined on both strands.

gag leader

N S G A S S G T A I G A H L F G V S P E
ATGTTTGGGAACTCTAGTGGACAGCATTGGGCTCATCTTTGGGGTCTCACTGAA -163

C R V L I G D E G A G P S K S L S E V S
TGCAGGGTGTGTGTCGAGAGGAGGAGGCCGACCTCTCTTCTGAGGTTCGA -103

F S V N Y O S R A A R L V I F C L V A S
TTTCGGTGTGGTACCAAGGCGCGCGACGCTCTGTCTATTCTGTGGTGGTCTCT -43

end gag leader → 0 — start gag p15
F L V P C L T F L I A E T V A S Q A
TTTCTGTCTGTCTTACCTTTTGAATTCGAGAACCTGATGGGCAACATATAGT -18

T P L S L T I D H M S E V R A R A H N Q
ACCCCTTAAGCTCAGCTTGTATCTGGTCTGAAGTCGGGCGACGACCATATCA -78

G V E V R K K K W V T L C E A E M V M
GGTGTCCAGGTCCGGAAGAAAGAAATGGTACCTATGTATGAGGCGAGTGGGTGATG -138

N V G M P R E G T F S L D N I S O V E K
AATGTGGGCTGGCCCCGAGGAAGAACTTTTCTCTGTATACATTTCCAGATTTGAGAA -198

K I F A P G P Y G H G D Q V P Y I T T M
AAGATCTTCGCCGGGACCTGTATGGACACCCGACCACTTCTATACATACCATGG -258

R S L A T D P P S M V R P F L P P K P
AGATCTTACGACAGACCCCTCTGTGGGTCTGCTCGATTTCTACCCCTCCCAAGCT -318

P T P L P Q P L S P Q P S A P L T S S L
CCACACCTCCCTCCACCTCTTTCGCGCGACCTCCGCGCTCTTACCTCTTCTCTC -378

15 → p12
T Y P L P K T D P P K P P V L P P D P S
TACCCCTTCTCCCAAGACAGACCTCCCAACCGCTGTGTGTACCGCTGATCTCTCT -438

S P L I D L L T E E P P Y P G G H G P
TCCCTTTAATGTCTCTTAACAGAAAGACCACTTCCATTCGCGGGGACAGGCA -498

L P S G P R T P T A S P I A S R I R
CTGCATAGTCTAGGACCAACCTTCCGCTTGTATGAGCGGTAGGAGAACGA -558

RENPAEESQALPREGPNRR
CGAGAACCTCTGAGATCTCAAGCCTCTCCCTTGAAGGAGGCCCAACACGA -618

P Q Y M P F S A S D L Y N M K S H R P
CCCCATGTGGCATCTTCACTGTATAGCTGTATAGTGAAGTGCATTAACCCCT -678

F S Q D P V A L T N L I E S I L V T H Q
TTCTCCAGAGACCTGGCCCTTACCTAATTAAGTTCATTTAGTGCATCA -738

P T M D D C Q Q L L A L L T G E E R Q
CCACCTGGGACACTGCCAGACGCTTCTGAGGAGCTCTGACAGCGGAGAGCA -798

R V L L E A R K Q V P G E D G R P T L Q
AGGTCTCTTGTAGGCGGGAAGAGGTTTCAAGCGGAGGAGGAGGCAACCCCACTA -858

P N V I D E T F P L T P R N M D F A T P
CCCATGTGATGATGAGATTTCCCTTACCGCTTCCAGCTGGGATTTGTACGCG -918

A G R E H L R L Y R Q L L A G L R G A
GAGGATGGACCTTCTATGCGAGTGTATAGGGTCTCCGCGGCT -978

end FeLV gag → start v-kit
A R R P T N L A Q V K Q V V E E I N G N
GCAAGACCCCACTATTTGGCAGATTAAGAGGTTTGTGAGGAGATTAATGAAC -1038

N Y I D P T Q L P Y D H K M E F P R N R
AATTACATAGACCAACAGCTTCTTACGATCAACAGGAGGATTTCCAGAACAG -1098

L S F G K T L G A G A F G K V V E A T A
CTGATTTTGGGAAACCTTGGGTGCCGTGCTTCCGGAAGGCTGGTGGAGGCCACTGCA -1158

Y G L I K S D A A M T V A V K M L K P S
TATGGCTGATTAAGTGGATGACGACCTACGCTTGTCTTAAGATGCTCAACCAAGT -1218

A H L T E A L M S E L V L S Y L G
GCCCATTAACCGAAGGAGGACCTCATGTCGAGCTCAAGTCTTGATTTACCTCGGC -1278

N H M N I V N L L G A C T V G G P T L V
AATCATAGATATTTGATTTCTCGGAGATGACCTGTGAGGCGCCACCTGCTGTC -1338

I T E Y C C Y G D L L N F L R R K R D S
ATTACGATGATTTGTGATGTTGATCTTTTGGTGAAGTGAACGATTCATCA -1398

F I C S K Q E D H A E V A L Y K N L L Q
TTTATTTGCTCAAGCAGGAAGATCATGAGAGATGGCCTTTATAGAACCTCTTCGAG -1458

S K E S S C N D S T N E Y M D M K P G V
TCAAGAGGATCTTCTGCAACGATAGTACTAATGATACATGACACATGAACCCGAGGT -1518

S Y V V P T K A D K R R S A R I G S Y I
TCTTATGTTGCCCAACGAGACCAAGGAGATCTGCCAGGATAGGCTCATACATA -1578

E R D V T A I M E D G E L A L D L E D
GAAAGATGATGACTCCGCCATCATGGAGCGGTGATGGCTGACCTAGGAGGAC -1638

L L S F S Y Q V A K G M A F L A S K M K
TTGCTGAGCTCTGTCACCGAGTGGCCAGGACCTGCGCTCTGCGCTGAAGATTTG -1698

I H R D L A A R N I L L T H G R I T K I
ATTCACGATAGTGGCTGCTAGAAATCTCTTACTACCTGCTGAATCAACAGAT -1758

C D F L A R D I K N D S N Y V A V K G N
TGTGATTTTGTCTAGCAGAGACATCAAGATGATTTATTTATGTTGCTCAAGGAAC -1818

A R L P V K M H A P E S I F N C V Y T F
GCTCGGCTACCTGAGTGGATGGCAGTGAAGATTTTCACTGTGTTGTACATATT -1878

E S D V M S Y G I F L W E L F S L G S
GAAAGTGTCTTGGCTATGGGATTTTCTGTGGGAGTGTCTCTTTAGGAAGAGC -1938

P Y P G M G V D S K F Y K M I K E G F R
CCCTACCTGGATGCTGACCTGATGTTCTACAGATGATGATGAGGAGGCTTCGA -1998

M L S P E H A P E M Y D I M K T C M D
ATGCTAGCCTGAGCAGCACTGCTGAATGTATGATCAATTAAGATGCTGCTGGAT -2058

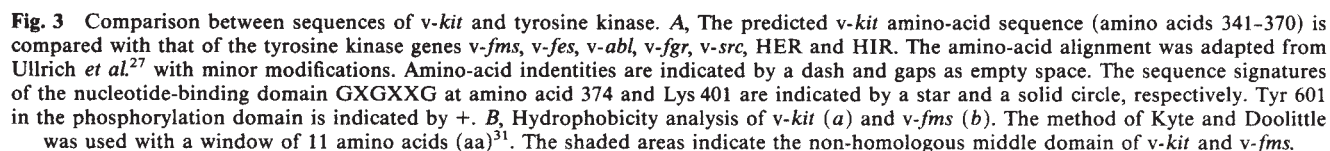
A D P L K R P T F K Q I V I Q L I E K Q
GCTGATCCCTGAAAGGCCAATTCAGAGATGCTGCGAGTATCTGAGAGAGCA -2118

end v-kit → FeLV pol
R K N E I T N R L T N
AATAAATGATTTACCTGCTTACCCACTGAATTTATAGAGGTCCTCAAGGCTCC -2178

ATGGGAATATGATGACGATGATTTAGACCTTGTGCAAAATCGAAGCTCATTAAGCC -2238

AAAAAGAGTACC 2251

3' Recombination Site in FeLV pol :
T A K K A A T E T H S S L T V L P T E L
ACAGCAAGAGAGGCCACAGAGACTTATCTAGCTTACCTCTTACCCACTGAATTT
I E G P K R — polymerase reading frame
ATAGAGGTCCTCAAGGCTCTATGGGA



On transfection of NIH 3T3 mouse cells, the cloned HZ4-FeSV provirus induced foci of transformed cells with an efficiency of $\sim 10^4$ focus-forming units (FFU) per pmol (ref. 20). Focus-forming virus could be obtained from the transformants by superinfection with amphotropic MuLV (10^3 – 10^4 FFU ml^{-1}), indicating that the cells contained the entire provirus. The morphology of the foci of transformed cells obtained by transfection with the pHZ4 DNA was indistinguishable from foci induced by HZ4-FeSV (amphotropic MuLV) pseudotype virus on NIH 3T3 cells (data not shown).

The relationship between the HZ4-FeSV *v-kit* sequences and those of the known avian and mammalian retroviral oncogenes was determined by hybridizing Southern blots containing the DNA of 18 different oncogenes with ^{32}P -labelled HZ4-FeSV DNA. No homology was found between HZ4-FeSV sequences and sequences of the oncogenes *h-ras*, *k-ras*, *n-ras*, *myc*, *myb*, *ski*, *fos*, *sis*, *src*, *yes*, *ros*, *abl*, *mos*, *fes*, *erb-A*, *erb-B*, *rel* or *raf* (ref. 21). However, a weak hybridization signal was detected with the 1.6-kb *Kpn*/*Bgl*III *v-fms* restricting fragment, although this hybridization was lost on washing at 65 °C in 0.2×SSC. As both *v-fms* and the HZ4-FeSV *v-kit* sequences are of feline origin, this indicates that HZ4-FeSV does not contain feline *v-fms* sequences. Furthermore, hybridization of blots containing HZ4-FeSV DNA with *v-fms* hybridization probes demonstrated that this homology included sequences of the *v-fms* kinase domain (not shown). Analysis of the immunological relationship between *v-kit* and *v-fms* by immunoprecipitation and SDS-PAGE revealed no immunoprecipitation with a polyclonal *fms*-specific antiserum, indicating that this reagent does not recognize determinants shared by *v-kit* and *v-fms* (Fig. 1*b*).

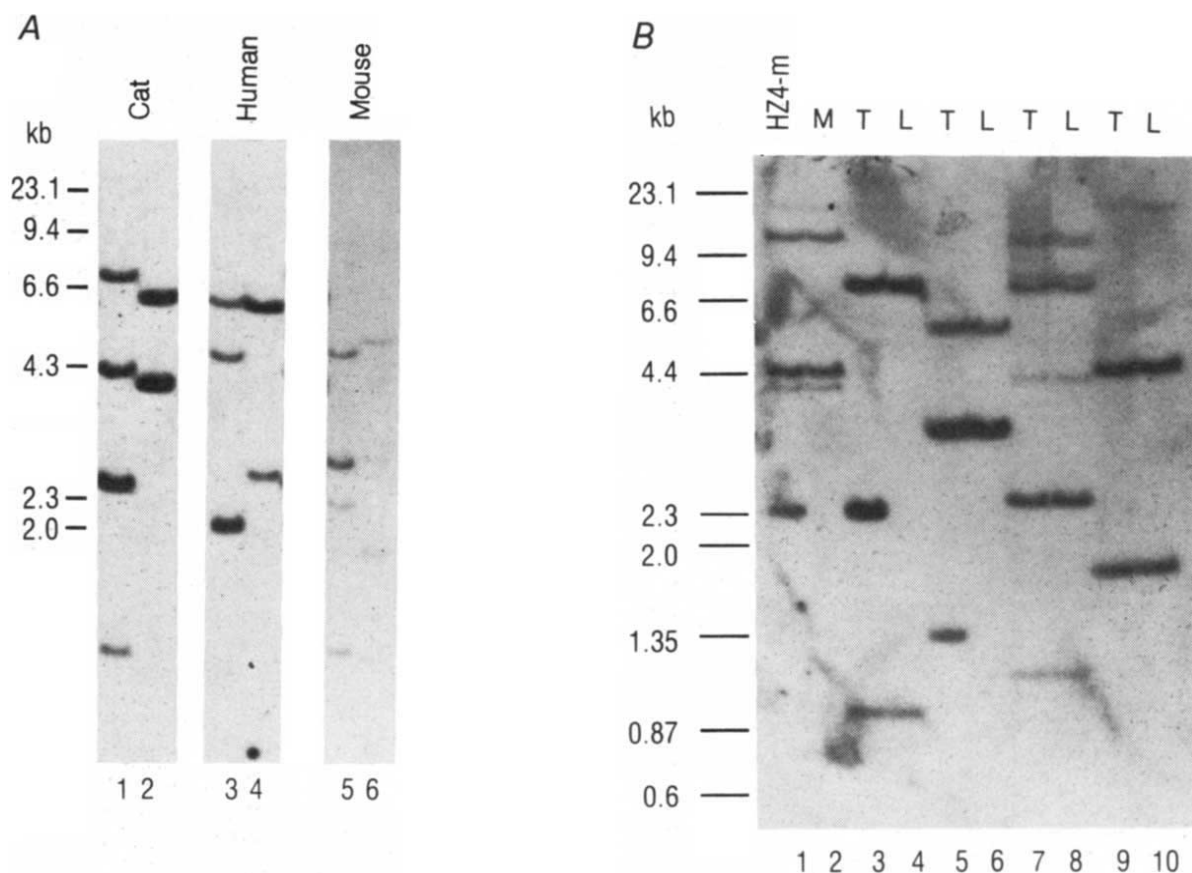


Fig. 4 *kit* sequences in normal DNA and in DNA from the tumour of cat 3637. **A**, Identification of *c-kit* sequences in normal mammalian DNAs. DNA from the kidney of cat 3590 was prepared from frozen tissue. The tissue (1 g) was homogenized in a Vitris homogenizer in TNE (10 mM Tris pH 8.0, 100 mM NaCl, 10 mM EDTA), nuclei were isolated by differential centrifugation and DNA was prepared from the nuclei as described previously². Aliquots (10 µg) of cat 3590 DNA, NIH 3T3 mouse DNA and human DNA (acute lymphocytic leukaemia line T-ALL-1) were digested with the restriction enzymes *Eco*RI (lanes 1, 3, 5) and *Hind*III (lanes 2, 4, 6) and fractionated in a 1% agarose gel. A Southern blot containing these DNAs was hybridized with a ³²P-labelled *v-kit* probe (2.75–3.5-kb *Sac*/*Sal* fragment) using non-stringent hybridization conditions. Blots were washed at 65 °C in 2×SSC. Migration of *Hind*III λ DNA fragments is indicated in kb. **B**, Identification of the HZ4-FeSV provirus in tumour DNA of cat 3637. DNA was prepared from tumour tissue (T) and from the liver (L) of cat 3637 and digested with restriction enzymes *Sac*I (lanes 9, 10), *Eco*RI (lanes 7, 8), *Bgl*II (lanes 5, 6) and *Kpn*I (lanes 3, 4), and DNA from HZ4-FeSV-infected (HZ4-m) and from uninfected mink cells (M) was digested with *Kpn*I (lanes 1, 2). Southern blots obtained after fractionation of these DNAs in a 1% agarose gel were hybridized with a ³²P-*v-kit* probe (2.75–3.5-kb *Sac*/*Sal* fragment). The blot was washed at 65 °C in 0.3×SSC. Migration of *Hind*III λ DNA and *Hae*III ΦX174 DNA fragments is indicated in kb.

Figure 2 shows the nucleotide sequence of the HZ4-FeSV *gag-kit* gene, determined using the dideoxy chain termination method of Sanger *et al.*²². An open reading frame extends from nucleotides –222 to 2,130 which includes 410 amino acids of FeLV *gag* origin, 370 amino acids of *kit* origin and 8 amino acids derived from the *pol* gene. To determine the 5' and 3' recombination sites between FeLV and *kit*, the *gag-kit* sequence was compared with the known FeLV *gag* sequence^{17,23}, a segment of FeLV *pol* and Moloney (M) MuLV *pol* sequences (Fig. 2). The 3' recombination site in FeLV *pol* corresponds to M-MuLV *pol* sequences 1.18 kb 5' of the *pol-env* junction²¹.

ST-, GA- and SM-FeSV as well as ST-FeLV encode *gag* leader sequences^{17,23}. However, only with SM-FeSV is this sequence thought to be of importance in the biosynthesis of the transforming protein. The predicted amino-acid sequence of *gag-kit* includes the FeLV *gag* leader, a stretch of 70 amino acids containing a hydrophobic signal peptide. The *gag-kit* protein is not glycosylated and probably does not include the leader sequence (H.W.S., unpublished). Therefore, initiation of translation may occur at the ATG at the beginning of the p15 gene at nucleotide 1.

The non-glycosylated *gag* gene products in FeLV are synthesized as a polyprotein of *M_r* 65K with the structure NH₂-p15-p12-p30-p10-COOH, which is post-translationally processed

into the individual peptides²⁴. The *gag*-derived segment of the HZ4-FeSV *gag-kit* protein consists of 340 amino acids, containing the total sequences of p15 and p12 but only 144 residues of p30. Compared with the sequences of ST-, GA- and SM-FeSV, the HZ4-FeSV *gag* sequences are slightly polymorphic, an unsurprising finding in view of the fact that all known FeSVs originate from naturally occurring tumours in pet cats.

The *v-kit* segment consists of 1,111 nucleotides. The open reading frame extends beyond the *kit* segment into the *pol* gene, where a TGA termination codon is reached 18 nucleotides 3' of the recombination site. The calculated *M_r* of 81.5K for the *gag-kit* polyprotein is in good agreement with the observed *M_r* 80K.

Comparison of the predicted *v-kit* amino-acid sequences with the known tyrosine-specific protein kinases (Fig. 3A) reveals good amino-acid homology between the *v-fms* sequences¹⁷ and *v-kit* (58% overall homology), particularly between amino acids 349–462 and 544–659. On the other hand, there is no obvious homology within amino acids 463–540. Homology also exists, although to a lesser extent, between *v-kit* and *v-fes*, *v-abl*, *v-fgr*, *v-src*, human epidermal growth factor receptor (HER) and human insulin receptor (HIR)^{16,18,25–28}. Lysine 401 as well as the configuration GXGXXG at amino acid 374 are characteristic of the nucleotide-binding domain of tyrosine-specific protein

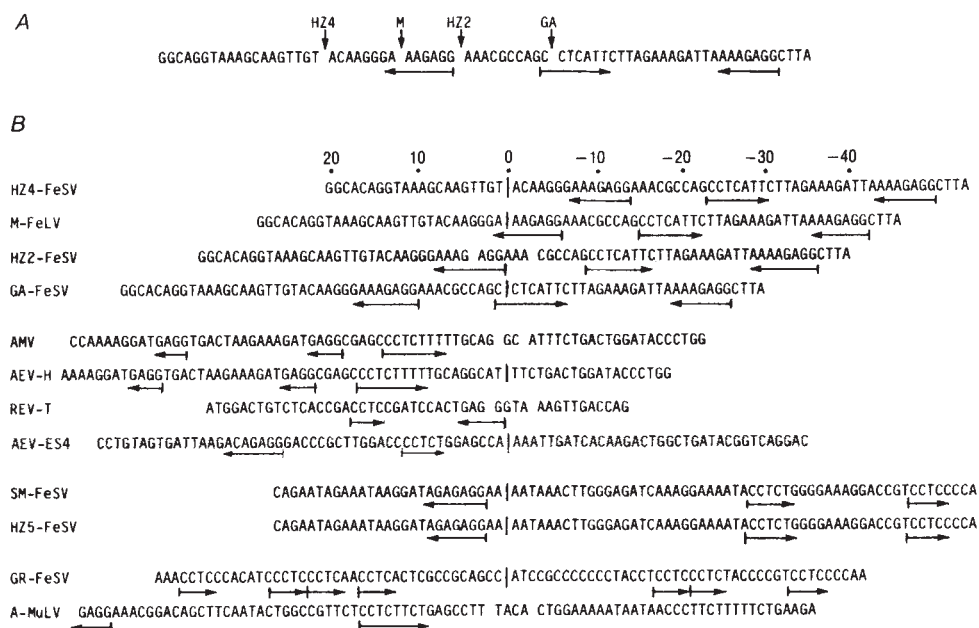


Fig. 5 Region of preferred 5' recombination sites in the FeLV *gag* p30 gene. **A**, The *gag* sequence shown is that found in GA-FeSV and SM-FeSV corresponding to nucleotides 1,568–1,638 (refs 23 and 17, respectively). The AAAGAGG sequence is indicated as $\leftarrow$, and the sequence CCTCTT as $\rightarrow$. **B**, GAGG and CCTC consensus sequences at 5'-recombination sites in acute transforming viruses. The retrovirus sequences at 5' recombination sites of the viruses indicated below are aligned. Homologies of retrovirus and *c-onc* sequences at the recombination site are set off by a space (for example, as in HZ2-FeSV). GAGG and CCTC consensus sequences are indicated as in **A**. The sequences at the 5' recombination site of the following viruses are shown: HZ4-FeSV (this paper), M-FeLV³², HZ2-FeSV (this paper), GA-FeSV¹⁶, SM-FeSV¹⁷, HZ5-FeSV (this paper), GR-FeSV¹⁸, Abelson murine leukaemia virus (A-MuLV)²⁵, AMV³⁶, AEV-H⁴⁸ and reticuloendotheliosis virus T (REV-T).

kinases^{29,30}. Furthermore, Tyr 601 is the homologue of the tyrosine phosphorylation site 416 of the Rous sarcoma virus *v-src* protein. Thus, *v-kit* has all the known structural features of a tyrosine-specific protein kinase. Attempts to identify a *gag-kit*-associated kinase activity have so far failed, and our structural analysis of *kit* suggests that this question should be investigated more carefully. The segment containing amino acids 468–542 does not have a counterpart in *v-fes*, *v-abl*, *v-fgr*, *v-src*, *v-yes*, HER or HIR, and this is consistent with the interpretation that the nucleotide-binding domain (amino acids 349–462) and the segment containing the phosphorylation site 601 (544–660) are separate functional domains. The corresponding middle domain of *v-fms* shows no significant amino-acid homology to *v-kit*, although the *v-kit* and *v-fms* middle domains are similarly hydrophilic in nature (Fig. 3B)³¹. It seems likely, then, that these domains are exposed at the exterior surface of the *v-kit* and *v-fms* proteins in the cytoplasm. As the middle domains of *v-kit* and *v-fms* are embedded between their catalytic domains, they may mediate substrate recognition and thus provide specificity for *c-kit*- and *c-fms*-mediated signal transduction.

Identification of *c-kit* in cellular DNA

Cellular DNA sequences homologous with the *v-kit* sequences of HZ4-FeSV were investigated by blot hybridization (Fig. 4A). In normal cat DNA, the *v-kit* probe (*Sac*/*Sal*, 0.7 kb) detected *Eco*RI and *Hind*III restriction fragments of 7.7, 4.3, 2.4, 1.2 kb and of 6.5, 4.0, 2.0 kb (weak signal, respectively). Human and mouse DNA shows a pattern of hybridization similar to that of cat DNA, the hybridization signal with human DNA being of almost equal intensity to the cat DNA restriction fragments. In contrast, hybridization to mouse DNA is somewhat weaker. These results suggest that there are cellular sequences in normal cat DNA which are homologous to *v-kit* and that these *c-kit* sequences are conserved in mammalian genomes. Hybridization of the *kit* probe detected restriction fragments corresponding to 12–15 kb of DNA, suggesting that *c-kit* is a large gene or perhaps that the *c-kit* sequences are derived from multiple loci.

Origin and oncogenic potential of HZ4-FeSV

To investigate the origin of HZ4-FeSV and its role in the generation of the fibrosarcoma in cat 3637, we used blot hybridization to determine whether the HZ4-FeSV provirus was contained in the tumour DNA (Fig. 4B). In both tumour and normal DNAs, the *v-kit* probe detected restriction fragments derived from *c-kit* and, using *Kpn*I and *Bgl*II, tumour-specific fragments of 2.3 and 1.3 kb, respectively, were seen. These fragments are virus internal fragments known from the molecularly cloned HZ4-FeSV provirus (Fig. 1). No tumour-specific restriction fragments were seen with enzymes known to generate fragments involving flanking cellular DNA sequences (*Eco*RI, *Sac*I, a second *Bgl*II fragment of large size would also be expected). The DNA from the tumour of cat 3637 therefore contains the HZ4-FeSV provirus. This not only demonstrates the origin of the virus, but is also consistent with the notion that HZ4-FeSV was involved in the generation of this tumour, although attempts to induce tumours by injection of kittens with HZ4-FeSV (FeLV) pseudotype virus have so far been unsuccessful. Our results also indicate that the tumour is polyclonal, presumably due to virus spread. If tumorigenesis in cat 3637 involved multiple stages, with HZ4-FeSV as an initiator, this did not result in visible clonal outgrowth, as has been seen in other systems^{51–53}. However, the virus could have been involved in a late stage of tumorigenesis.

Preferred recombination site in FeLV *gag*

The 5' junction between the FeLV and *kit* sequences in HZ4-FeSV occurs at nucleotide 1,020 of the FeLV *gag* gene^{17,23}. Comparison of this junction with those in GA-FeSV, HZ2-FeSV and the M-FeLV *myc* virus^{16,32} revealed that the 5' recombination sites of these four viruses are clustered within a region of 24 bp in the FeLV *gag* gene (Fig. 5A). This 24-bp region in FeLV p30 therefore defines a preferred region for recombination which is involved in the generation of acute transforming feline retroviruses.

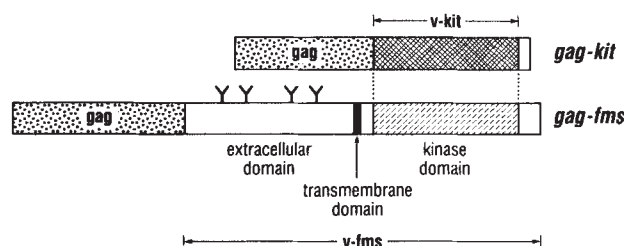


Fig. 6 Schematic comparison of the *gag-kit* and *gag-fms* transforming proteins. Glycosylation sites in the extracellular domain of *v-fms* are indicated by Y.

It seemed possible that the sequences in the FeLV p30 region facilitate the generation of the 5' *gag-*onc** junctions and we therefore inspected the nucleic acid sequence around the recombination sites in FeLV p30 to identify sequence motifs characteristic of this region. A heptanucleotide AAAGAGG occurs twice, at nucleotides 1,026 and 1,064, and the complement of this sequence (CCTC(T)TT) occurs once, at nucleotide 1,042, indicating sequence symmetry and potential for secondary structure formation (Fig. 5A). The GAGG and CCTC consensus sequences are found at the 5' junctions of at least 16 of the 28 characterized virus strains, suggesting some link between this sequence and the recombination event (Fig. 5B). Of these viruses, AEV-H and avian myeloblastosis virus (AMV) have junctions that are 2 nucleotides apart and HZ5-FeSV, a new *fms*-containing virus, and SM-FeSV have identical junctions (P.B. and P.C.G., unpublished) (Fig. 5B). In plasmacytomas involving *c-myc* chromosomal translocations, the GAGG sequence motif has been found adjacent to the translocation breakpoints³³. Thus, events leading to the generation of oncogene-containing retroviruses and chromosomal translocations may involve common recombination mechanisms.

With many acute transforming viruses, the recombinations at the 5' retrovirus-*v-*onc** junctions must result in functional gene fusions, and constraints for expression and function of the transforming proteins may dictate the formation of these junctions. On the other hand, recombination sites in *gag-*onc** fusions are known to occur in various regions of the *gag* gene, and *in vitro* alteration of the placement of *onc* sequences within retroviruses can be achieved without loss of function^{34,35}. In addition, the recombinations in HZ4-, HZ2- and GA-FeSVs and in M-FeLV involve four different oncogenes. Our observations therefore suggest that the formation of 5' *gag-*onc** junctions, which is an early step in the generation of acute transforming viruses^{36,37}, may be facilitated by at least two factors, recombinogenic sequences and short sequence homologies between the parental strands at the recombination site described previously³⁸.

Discussion

The present studies demonstrate that HZ4-FeSV is an acute transforming feline retrovirus which, in analogy with other acute transforming viruses, was generated by transduction of feline *c-*onc** sequences (which we have designated *kit*). HZ4-FeSV has the structure 5' Δ*gag-kit*-Δ*pol*-Δ*env* 3', and the only gene product we have identified in HZ4-FeSV-infected cells is an 80K protein that contains determinants of FeLV *gag* p15, p12 and p30 as well as *kit* sequences. The *v-kit* amino-acid sequences revealed homology with the tyrosine kinase gene family, its closest relative being *v-fms*.

The *kit* gene resembles *v-fms* not only in significant amino-acid homology, but also in the very distinct domain structure of the protein. *v-fms* has recently been found to have the features of a transmembrane receptor, that is, it consists of an outer cellular domain, a transmembrane domain and an inner cellular domain containing a tyrosine-specific protein kinase; in fact, it

is homologous with the CSF-1 receptor^{39,40}. The *v-fms* sequence is 2.9 kb long, while *v-kit* is 1.1 kb. The *v-kit* segment corresponds to *v-fms* sequences of the inner cellular domain, and does not contain a transmembrane domain or sequences of an outer cellular domain. Retroviral oncogenes are thought to represent segments of *c-*onc** mRNAs, and it is likely that *v-kit* is only a part of the *c-kit* mRNA; significant portions of *c-kit* coding sequences are probably missing in *v-kit* on both the 5' and 3' sides, although the nature of these sequences is not known. Because of the close relationship between *v-kit* and *v-fms*, it is reasonable to assume that *c-kit* and *c-fms* are closely related genes. Like *c-fms*, then, *c-kit* could encode a transmembrane receptor that is involved in signal transduction.

Most protein kinase oncogenes need to be associated with cellular membranes for function^{41,42}, and two different kinds of association are known, association via myristic acid⁴³ and anchorage in membranes by means of a transmembrane domain³⁹. The 65K *gag* polypeptide of mammalian retroviruses and the *gag-*onc** fusion proteins of mammalian acute transforming viruses are myristylated at their N-terminus and are thus associated with cellular membranes⁴⁴. As *v-kit* does not have a transmembrane domain like *v-fms*, we predict that, in analogy with other *gag-*onc** transforming proteins, the *gag-kit* protein is associated with cellular membranes via *gag*-linked myristylation.

Many different oncogenes have been identified in acute transforming viruses isolated from fibrosarcomas of animals infected by retroviruses. Interestingly, the oncogenes *src*, *yes* and *ros* known from avian retroviruses have not been found in mammalian retroviruses. *kit*, *fms* and *fgr*, on the other hand, have been found only in viruses isolated from feline fibrosarcomas. This inherent specificity of transduction of *c-*onc**s by FeLV may be due to recombinogenic sequences in the retrovirus and the *c-*onc**s, as well as functional constraints for the expression of the transforming proteins. It has been proposed that integration of a retrovirus into the cellular DNA may be restricted to transcriptionally active regions of the chromosome^{45,46}. Thus, the tissue tropism of a particular virus strain would determine the *c-*onc**s that can be transduced. Different tissue tropisms of FeLV and avian leukemia virus could therefore also explain the different transduction specificities.

We thank O. Jarrett for the FeLV subgroup determinations; C. Sherr, K. C. Robbins, U. Rapp, I. Verma, S. P. Goff, D. Dina, M. Wigler, R. Ellis, J. M. Bishop, H. Temin and M. Yoshida for DNA reagents used in this study; E. Stavnezer and L. H. Wang for providing unpublished reagents; C. Payne for preparation of numerous plasmids; M. S. Lee for help with the analysis of the tumour DNA; and M. Singhal, K. Brown and R. Markovich for technical assistance. This work was supported by grants from the American Cancer Society (MV-82) and the NIH (CA-32926) to P.B. and (CA-16599) to W.D.H. and H.W.S. H.W.S. was a Scholar of the Leukemia Society of America. P.J.B. was a Horsfall awardee.

Received 16 October 1985; accepted 13 January 1986.

- Devare, S. G. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **79**, 3179-3182 (1982).
- Besmer, P., Snyder, H. W. Jr, Murphy, J. E., Hardy, W. D. Jr & Parodi, A. J. *Virology* **46**, 606-613 (1983).
- Waterfield, M. D. *et al.* *Nature* **304**, 35-39 (1983).
- Downward, J. *et al.* *Nature* **307**, 521-527 (1984).
- Sherr, C. J. *et al.* *Cell* **41**, 665-676 (1985).
- Hardy, W. D. Jr, Old, L. J., Hess, P. W., Essex, M. & Cotter, S. *Nature* **244**, 266-269 (1973).
- Besmer, P. *Curr. Topics Microbiol. Immun.* **107**, 1-27 (1984).
- Hardy, W. D. Jr, Theilen, G. H. *Nature* **221**, 1074-1075 (1969).
- Gardner, M. B. *et al.* *Nature* **226**, 807-809 (1970).
- McDonough, S. K., Larsen, S., Brodey, R. S., Stock, W. D. & Hardy, W. D. Jr *Cancer Res.* **31**, 953-956 (1971).
- Irgens, K., Wyers, M., Moraillon, A., Parodi, A. & Fortuny, V. C. *r. hebdom. Séanc. Acad. Sci., Paris* **276**, 1783-1786 (1973).
- Rasheed, S., Barbacid, M., Aaronson, S. A. & Gardner, M. B. *Virology* **117**, 238-244 (1982).
- Snyder, H. W., Singhal, M. C., Zuckerman, E. E. & Hardy, W. D. *Virology* **132**, 205-210 (1984).
- Ziemiecki, A. *et al.* *Virology* **138**, 324-331 (1984).
- Besmer, P., Hardy, W. D. Jr, Zuckerman, E., Lederman, L. & Snyder, H. S. *Nature* **303**, 825-828 (1983).

16. Hampe, A., Laprevotte, I., Galibert, F., Fedele, L. A. & Cherr, C. J. *Cell* **30**, 775-785 (1982).
17. Hampe, A., Gobet, M., Sherr, C. J. & Galibert, F. *Proc. natn. Acad. Sci. U.S.A.* **81**, 85-89 (1984).
18. Naharro, G., Robbins, K. C. & Reddy, E. P. *Science* **223**, 63-66 (1984).
19. Sarma, P. S. & Log, T. *Virology* **54**, 160-169 (1973).
20. Graham, E. L. & van der Eb, A. J. *Virology* **52**, 456-467 (1973).
21. Weiss, R., Teich, N., Varmus, H. & Coffin, J. *Molecular Biology of Tumor Viruses* (Cold Spring Harbor Laboratory, New York, 1982).
22. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463 (1977).
23. Laprevotte, I., Hampe, A., Sherr, C. J. & Galibert, F. *J. Virol.* **50**, 884-894 (1984).
24. Okazinski, G. F. & Velicer, L. F. *J. Virol.* **22**, 74-85 (1977).
25. Reddy, E. P., Smith, M. J. & Srinivasa, A. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3623-3627 (1983).
26. Schwartz, D. E., Tizard, R. & Gilbert, W. *Cell* **32**, 853-869 (1983).
27. Ullrich, A. *et al. Nature* **313**, 756-761 (1985).
28. Ullrich, A. *et al. Nature* **309**, 418-425 (1984).
29. Wierenga, R. K. & Hol, W. G. T. *Nature* **302**, 842-844 (1983).
30. Zoller, M. J., Nelson, N. C. & Taylor, S. S. *J. biol. Chem.* **256**, 10837-10842 (1981).
31. Kyte, J. & Doolittle, R. F. *J. molec. Biol.* **157**, 105-132 (1982).
32. Braun, M. J., Deininger, P. L. & Casey, J. W. *J. Virol.* **55**, 177-183 (1985).
33. Piccoli, S. P., Caimi, P. G. & Cole, M. D. *Nature* **310**, 327-330 (1984).
34. Foster, D. A. & Hanafusa, H. *J. Virol.* **48**, 744-751 (1983).
35. Prywes, R., Hoag, T., Rosenberg, N. & Baltimore, D. *J. Virol.* **54**, 123-132 (1985).
36. Klemmner, K.-H., Gonda, T. J. & Bishop, J. M. *Cell* **31**, 453-463 (1982).
37. Goldfarb, M. & Weinberg, R. *J. Virol.* **38**, 136-150 (1981).
38. Wang, J. Y. J. *et al. Cell* **36**, 349-356 (1984).
39. Rettenmier, C. W. *et al. Cell* **40**, 971-981 (1985).
40. Manger, R., Najita, L., Nichols, E. J., Hakomori, S.-I. & Rohrschneider, L. *Cell* **39**, 327-337 (1984).
41. Pellman, D., Garber, E. A., Cross, F. R. & Hanafusa, H. *Nature* **314**, 374-377 (1985).
42. Roussel, M. F., Rettenmier, C. W., Look, A. T. & Sherr, C. J. *Molec. cell. Biol.* **4**, 1999-2009 (1984).
43. Schultz, A. M., Henderson, L. E., Oroszlan, S., Garber, E. A. & Hanafusa, H. *Science* **227**, 427-429 (1985).
44. Schultz, A. M. & Oroszlan, S. *J. Virol.* **46**, 355-361 (1983).
45. Breindl, M., Habers, K. & Jaenisch, R. *Cell* **38**, 9-16 (1984).
46. Schubach, W. & Groudine, M. *Nature* **307**, 702-708 (1984).
47. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
48. Yamamoto, T. *et al. Cell* **35**, 71-78 (1983).
49. Yanisch-Perron, C., Vieira, T. & Messing, T. *Gene* **33**, 103-119 (1985).
50. Wilhelmsson, K. C., Eggleston, K. & Temin, H. J. *J. Virol.* **52**, 172-182 (1984).
51. Neel, B. G., Hayward, W. S., Robinson, H. L., Fang, J. & Astrin, S. M. *Cell* **23**, 323-334 (1981).
52. Fung, Y. K. T., Lewis, W. G., Crittenden, L. B. & Kung, H. J. *Cell* **33**, 357-368 (1983).
53. Nusse, R., van Oyen, A., Cox, D., Fung, Y. K. T. & Varmus, H. *Nature* **307**, 131-136 (1984).

LETTERS TO NATURE

Limits of X-ray variability in active galactic nuclei

P. Barr

Exosat Observatory, European Space Operations Centre,
Robert Bosch Str. 5, 6100 Darmstadt, FRG

R. F. Mushotzky

Laboratory for High Energy Astrophysics,
NASA Goddard Space Flight Center, Greenbelt,
Maryland 20771, USA

Active galactic nuclei (AGN) have been observed to vary in all wavebands on timescales ranging from years to minutes¹⁻³. The timescale of variability can yield information about the efficiency of the emission mechanism⁴ and the details of the accretion process⁵. Most of the luminosity of Seyfert galaxies and quasars probably occurs in the hard X-ray region, with a cutoff near 1 MeV (ref. 6). (Although there have been reports of high luminosity 'ultraviolet bumps', optical observations of a large sample of objects have shown these to be rare^{7,8}.) We show here that there is a significant correlation between the X-ray luminosity and timescale of X-ray variability for Seyfert galaxies and quasars. We interpret this as evidence that the emitting plasma is near the limit of being dominated by electron-positron pairs. BL Lac objects do not follow this pattern; this may be due either to relativistic beaming or to the differing importance of the pair production process.

We have compiled from the literature a sample of Seyfert galaxies and quasars for which the X-ray variability is reliably established, is the fastest variability reported for that particular object, and is time-resolved. Table 1 lists, for each object, name, intensity doubling timescale, 2-10 keV luminosity and object class. Since the temporal behaviour is adequately sampled, the errors in the doubling time are small. Only those sources seen to vary by more than 10% were included. In addition, a number of BL Lacs are included. Because variability in the X-ray emission of BL Lac objects has been less widely reported than in Seyferts, we have dropped the requirement that the BL Lac variations be time-resolved in order to obtain an adequate sample. Figure 1 is a log-log plot of the mean 2-10 keV rest-frame luminosity (assuming Hubble constant $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and deceleration parameter $q_0 = 0$), L_x , against the time for the source intensity to double, Δt . For Mkn205, OX169 and MCG5-23-16, the amplitude of variation is less than a factor of two and Δt is extrapolated linearly from the observed variability;

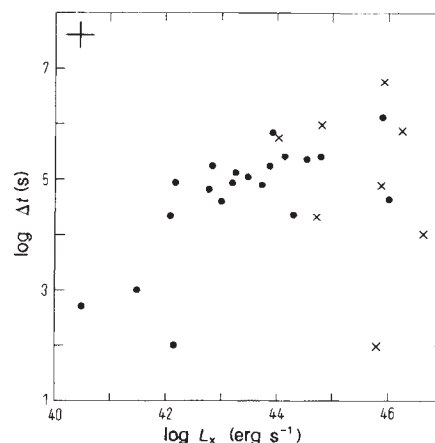


Fig. 1 Plot of $\log \Delta t$ against $\log L_x$ for Seyfert galaxies and QSOs (circles) and BL Lac objects (crosses). A typical error bar is shown in the upper left-hand corner.

the other objects exhibited variability by factors between two and six. A typical error bar is shown in Fig. 1. For OX169 and Mkn205, L_x is extrapolated from the soft X-ray band assuming a power-law spectrum with energy index 0.7 (ref. 9) and purely galactic absorption¹⁰; the other data are all direct measurements. The BL Lac luminosities are for the 0.5-3.5 keV band, except for Mkn205 (2-17 keV) and 0323+023 (1-20 keV). The 1E1402.3+0416 point represents the lower limit to its luminosity.

For Seyferts and quasars, we find a highly significant correlation between $\log L_x$ and $\log \Delta t$, of the form $\log L_x = a \log \Delta t + b$ erg s^{-1} , with $a = 0.9 \pm 0.2$, $b = 39 \pm 1$ and linear correlation coefficient $r_c = 0.7$. The probability of achieving this by chance from a random sample is $P < 10^{-3}$ (exclusion of a single point, the Ariel V observation of 3C373, reduces P to $10^{-5.5}$). This trend is unlikely to be an observational selection effect; a linear regression of the logarithm of the 2-10 keV intensity against $\log \Delta t$ yields $r_c = 0.32$, while for signal-to-noise ratio versus $\log \Delta t$, $r_c = 0.2$. On the other hand, the BL Lac objects obey no such relationship. A linear regression yields correlation coefficient $r_c = 0.21$.

We consider first the Seyferts and quasars. Assuming that their hard X-ray spectrum extends to 1 MeV (ref. 6) with energy spectral index 0.7 (ref. 9), a bolometric correction of 1.0 to $\log L_x$ is indicated. Within the errors the correlation between L_x and Δt is linear. The hard X-ray luminosity for this sample, L , may thus be written $\log L = \log \Delta t + 40 \text{ erg s}^{-1}$.

How valid is this treatment? The most homogeneous sampling performed so far has been that by Tennant and Mushotzky¹¹,

Table 1 Doubling time and luminosity of selected Seyfert galaxies, quasars and BL Lac objects

Source	log Δt	log L_x	Ref.	Object class
NGC6814	2.00	42.15	11	Seyfert
NGC3031	2.70	40.48	18	Seyfert
NGC4051	3.0	41.48	15	Seyfert
NGC3227	4.33	42.08	11	Seyfert
OX169	4.36	44.30	12	QSO
NGC4151	4.60	43.00	17	Seyfert
3C273	4.65	46.10	13	QSO
NGC7582	4.81	42.78	30	Seyfert
NGC7469	4.90	43.73	13	Seyfert
NGC2110	4.94	43.20	30	Seyfert
NGC5128	4.94	42.17	31	Radio galaxy
MGC5-23-16	5.03	43.48	11	Seyfert
NGC2992	5.11	43.26	13	Seyfert
NGC5506	5.34	42.83	30	Seyfert
NGC526a	5.24	43.87	13	Seyfert
Mkn205	5.35	44.54	12	QSO
3C382	5.41	44.77	*	BLRG
4U0241+62	5.41	44.13	13	QSO
Mkn841	5.84	43.92	32	QSO
1H1820+643	6.11	45.87	33	QSO
PKS0548-32	6.00	44.81	34	BL Lac
3C371	5.78	44.03	35	BL Lac
PKS2155-304	4.90	45.87	36	BL Lac
OJ287	6.78	45.93	37	BL Lac
3C66A	5.88	46.26	38	BL Lac
1E1402.3+0416	4.03	46.63	28	BL Lac
Mkn421	4.33	44.72	13	BL Lac
0323+023	2.00	45.81	29	BL Lac

Δt , Time for source intensity to double; L_x , mean 2–10 keV rest-frame luminosity.

* P.B. *et al.*, unpublished data.

using HEAO-1 A2 observations of 38 AGN ranging over four decades in intrinsic luminosity. All of their sources for which variability was detected exhibited timescales consistent with our observed correlation. Furthermore, the doubling timescale in individual objects is repeatable at different epochs. The low-amplitude variations of 3C273 found by Einstein Observatory observations¹² imply a doubling timescale similar to that seen in a large-amplitude flare by Ariel V¹³; Einstein¹⁴ and Exosat¹⁵ observations of NGC4051 suggest similar Δt , as do the Ariel V¹⁶ and HEAO-1¹⁷ observations of NGC4151. There are some objects which have not been observed to vary, but AGN are known to experience quiescent epochs between episodes of variability^{17,18}. The duty cycle of variability is very poorly determined and it is entirely possible that more extensive observations of the 'steady' AGNs may reveal variability.

We know of only one observation of a quasar which diverges strongly from our suggested doubling time–luminosity relationship. This is the remarkable behaviour¹⁹ of 1525+227, which doubled its intensity (initially 3×10^{44} erg s⁻¹) in 200 s (a 4 σ result). If real, this would necessitate relativistic beaming to avoid efficiencies of >100%. (Although Bassani *et al.*²⁰ have attempted a similar treatment, their sample is highly heterogeneous in that the variability timescales used were often taken from infrared, optical or ultraviolet flux measurements and even from optical polarization variations. It is by no means evident that these timescales are relevant to the hard X-ray band in which most of the luminosity arises; indeed, in Seyfert galaxies there is strong evidence that the emission in different wavebands may arise in quite distinct components²¹.)

In the absence of relativistic bulk motions, the characteristic time for the intensity to double must be constrained by the characteristic source size R . We naively assume $R \leq c \Delta t$; then $\log L/R \geq 29.6$ erg cm⁻¹ s⁻¹. Defining the dimensionless compactness parameter²² $\lambda = L\sigma_T/Rm_e c^3$ (where σ_T is the Thomson cross-section) we have, for the Seyferts and quasars, $\lambda \geq 10$. In this regime electron–positron pair production by two-photon

annihilation is important. For $\lambda > 4\pi$ the plasma would be dominated by the pairs²²; the compactness parameter cannot be much larger than this because at $\lambda > 30$ the pairs strongly modify the emergent X-ray spectrum, producing a much steeper soft X-ray continuum than that observed²³. It is therefore likely that the emitting plasma in this sample of AGN are marginally dominated by electron–positron pairs.

Araki and Lightman²⁴ have shown that a thermal plasma can be relativistic ($kT_e \geq 3m_e c^2$) only if $\lambda < 0.15(kT_e/m_e c^2)^{-(1+4\alpha)}$, where α is the spectral index of the emergent X-rays. Since $\alpha \approx 0.7$, a relativistic thermal plasma would have $\lambda < 0.01$, inconsistent with the observations. Therefore, if the plasma is thermal, its temperature is not highly relativistic. If the pairs are nonthermal, Zdziarski and Lightman²⁵ have shown that, for this likely compactness parameter, inverse Compton scattering of soft photons will produce the hard ($\alpha \approx 0.7$) X-ray power-law spectrum observed.

The Thomson optical depth due to pairs is²⁶ $\tau \approx (f_g \lambda)^{1/2}$, where f_g is the fraction of primary luminosity radiated above $2m_e c^2$. For $\lambda \approx 10$ and a primary photon spectrum with an upper cutoff near 1 MeV, $\tau \approx 1$. The efficiency of conversion of matter to energy⁴, η , is $\geq 5 \times 10^{-43} L/\Delta t$; that is, $\geq 0.5\%$, where the inequality disappears at $\tau = 1$.

Since $L/R < L_{\text{Edd}}/R_s$, where L_{Edd} is the Eddington limit and R_s the Schwarzschild radius, a further constraint is $L > 10^{-3} L_{\text{Edd}}$ (for the classical Eddington limit). However, if the plasma is pair-dominated, L_{Edd} is reduced by $m_p/m_e = 1836$, the plasma is very near the Eddington limit and pair effects may dominate the hard photon flux for these objects²³.

If the X-ray emission arises at the surface of an optically thick photosphere, the emissivity $\epsilon = L/4\pi R^2$, so that Fig. 1 may be considered to be a plot of luminosity against emissivity, like the Hertzsprung–Russell diagram. For accretion onto a central object of mass M with R/R_s fixed for the entire sample, Fig. 1 implies that $\epsilon \propto 1/M$ and the luminosity per unit mass is constant.

Finally, we note that there is one class of AGN which consistently violate the Seyfert/QSO L -versus- Δt relationship; these are the BL Lac objects. For such objects, $L/\Delta t$ can be far lower than that implied by the optically thick pair-production limit. White *et al.*²³ have suggested that BL Lacs are in fact AGN with such high luminosities that the emitting plasma is severely pair-dominated, with $\lambda > 30$, such that any γ -rays and high energy X-rays are reprocessed to lower energies by two-photon annihilation (and by Compton scattering off the pairs), causing the steep soft X-ray spectra observed.

Conversely, if the observed spectrum of BL Lac objects represents their primary photon spectrum, then, when extrapolated to above 511 keV, it may not yield enough γ -rays to generate pairs; although there are no measurements of the X-ray spectra of BL Lacs above ~ 20 keV, the observed 2–10 keV spectra appear generally quite soft²⁷. In addition, the large optical depths implied by the severely pair-dominated case may be difficult to reconcile with the observed rapid variability. Another possibility is that geometric effects (relativistic bulk motions and anisotropic emission) may obviate the necessity for large values of λ (and associated copious pair production), and in fact are necessary to explain the rapid variability of (and implied high efficiencies in) 1E1402.3+0416 (ref. 28) and 0323+023 (ref. 29). Interestingly, exclusion of the three radio-loud sources in the Seyfert/QSO sample leads to a considerable improvement in the goodness of the fit (from 99.90% to 99.99% significance). It is tempting to speculate that relativistic beaming and/or anisotropic emission mechanisms are also relevant to the X-ray emission from radio-loud quasars, although the evidence so far is, at best, highly circumstantial.

P.B. is affiliated to the Astrophysics Division, Space Science Department, European Space Agency. R.F.M. thanks the Institute of Astronomy, Cambridge, for hospitality extended during 1985.

Note added in proof: Recent EXOSAT observations of the Seyfert galaxies NGC3516 (G. Reichert, personal communication), Mkn766 and NGC4593 (P.B. *et al.*, unpublished data) have revealed X-ray variability fully consistent with the suggested doubling time–luminosity relationship.

Received 26 November 1985; accepted 5 February 1986.

1. Mchardy, I. *Space Sci. Rev.* **40**, 559–584 (1985).
2. Barr, P. *et al. Mon. Not. R. astr. Soc.* **193**, 549–562 (1980).
3. Glass, I. S. *Mon. Not. R. astr. Soc.* **197**, 1067–1080 (1981).
4. Fabian, A. C. & Rees, M. in *X-ray Astronomy: Proc. Symp. 21st Plenary Meeting of Cospar* (eds Baity, W. A. & Peterson, L. E.) 381–396 (Pergamon, Oxford, 1979).
5. Abromowicz, M. & Nobili, L. *Nature* **300**, 506–507 (1982).
6. Bassani, L. & Dean, A. *Astr. Astrophys.* **122**, 83–87 (1983).
7. Malkan, M. & Sargent, W. L. *Astrophys. J.* **254**, 22–37 (1982).
8. Richstone, D. O. & Schmidt, M. *Astrophys. J.* **235**, 361–376 (1980).
9. Rothschild, R. E. *et al. Astrophys. J.* **269**, 423–437 (1983).
10. Heiles, C. *Astr. Astrophys. Suppl.* **20**, 37–56 (1975).
11. Tennant, A. F. & Mushotzky, R. F. *Astrophys. J.* **264**, 92–104 (1983).
12. Zamorani, G., Giommi, P., Maccacaro, T. & Tannanbaum, H. *Astrophys. J.* **278**, 28–36 (1984).
13. Marshall, N., Warwick, R. & Pounds, K. *Mon. Not. R. astr. Soc.* **194**, 987–1002 (1981).
14. Marshall, F. E., Holt, S. S., Mushotzky, R. F. & Becker, R. H. *Astrophys. J.* **269**, L31–L37 (1983).
15. Lawrence, A., Watson, M. G., Pounds, K. A. & Elvis, M. *Mon. Not. R. astr. Soc.* **217**, 685–699 (1985).
16. Lawrence, A. *Mon. Not. R. astr. Soc.* **192**, 83–94 (1980).
17. Mushotzky, R. F., Holt, S. S. & Serlemitsos, P. *Astrophys. J.* **225**, L115–L118 (1978).
18. Barr, P., Giommi, P., Wamsteker, W., Gilmozzi, R. & Mushotzky, R. F. *Bull. Am. astr. Soc.* **17**, 608 (1985).
19. Matilsky, T., Schrader, C. & Tannanbaum, H. *Astrophys. J.* **258**, L1–L8 (1983).
20. Bassani, L., Dean, A. J. & Sembay, S. *Astr. Astrophys.* **125**, 52–58 (1983).
21. Tanzi, E. *et al. ESA scient. Publ.* **218**, 111–117 (1984).
22. Svensson, R. in *X-Ray and UV Emission from Active Galactic Nuclei* (eds Brinkman, W. & Trumper, J.) 152–163 (MPE Rep. 184, Munich, 1984).
23. White, N. E., Fabian, A. C. & Mushotzky, R. *Astr. Astrophys.* **133**, L9–L11 (1983).
24. Araki, A. & Lightman, A. P. *Astrophys. J.* **269**, 49–58 (1983).
25. Zdziarski, A. A. & Lightman, A. P. *Astrophys. J.* **294**, L79–L85 (1985).
26. Guilbert, P., Fabian, A. & Rees, M. *Mon. Not. R. astr. Soc.* **205**, 593–604 (1983).
27. Urry, M. & Mushotzky, R. F. *Astrophys. J.* **253**, 38–48 (1982).
28. Giommi, P. *et al. Astrophys. J.* **303** (in the press).
29. Doxsey, R. *et al. Astrophys. J.* **264**, L43–L48 (1983).
30. Mushotzky, R. F. *Astrophys. J.* **256**, 92–102 (1983).
31. Lawrence, A., Pye, J. P. & Elvis, M. *Mon. Not. R. astr. Soc.* **181**, 93P–99P (1977).
32. Arnaud, K. *et al. Mon. Not. R. astr. Soc.* **217**, 105–113 (1985).
33. Snyder, W. A. & Wood, K. in *X-Ray and UV Emission from Active Galactic Nuclei* (eds Brinkman, W. & Trumper, J.) 38–47 (MPE Rep. 184, Munich, 1984).
34. Urry, C. M., Mushotzky, R. F., Kondo, Y., Hackney, K. & Hackney, L. *Astrophys. J.* **261**, 12–18 (1982).
35. Snyder, W. A. *et al. Astrophys. J.* **259**, 38–47 (1982).
36. Snyder, W. A. *et al. Astrophys. J.* **237**, L11–L14 (1980).
37. Worrall, D. *et al. Astrophys. J.* **261**, 403–411 (1982).
38. Maccacagni, D., Maraschi, L., Tanzi, E. G., Tarengini, M. & Chiappetti, L. *Astrophys. J.* **273**, 75–80 (1983).

Detection of the very hot central star in NGC2440

P. D. Atherton*‡, N. K. Reay† & S. R. Pottasch*

* Kapteyn Astronomical Institute, University of Groningen, PB 800, 9700-AV Groningen, The Netherlands
† Astrophysics Group, Blackett Laboratory, Imperial College, London SW7 2BZ, UK

It has been argued¹ that the extremely faint central stars of some planetary nebulae must be very hot, with most of their energy output in the ultraviolet. We now report the detection of the central star of the planetary nebula NGC2440 in a narrow-band continuum image using a CCD (charge-coupled device) camera at the prime focus of the Anglo-Australian telescope. Its visual magnitude has been measured as 18.9 ± 0.2 . Its Zanstra temperature^{2,3} is about 350,000 K, so that it is one of the hottest stars ever observed. Its radius corresponds to that of a nearly degenerate star. Theoretical calculations can explain the observed temperature and luminosity if it has a mass of at least $1.0 M_{\odot}$ and is in its cooling stage, but the predicted age of the nebula is considerably less than that required by the calculations. Furthermore, the present estimates of the progenitor mass of a $1-M_{\odot}$ white dwarf are brought into question.

For such a star, all the energy in the Lyman continuum (at wavelengths $< 912 \text{ \AA}$) will go into ionizing the surrounding nebular gas, with the energy reappearing at longer wavelengths as recombination and collisionally excited lines. It is possible to estimate the number of ionizing photons emitted by the star because each Lyman photon will (eventually) result in one Balmer photon. The temperature of the central star (the Zanstra temperature) is computed from the ratio of the number of H β photons to the visual continuum emission, assuming either black-body radiation or a model atmosphere. While the total Balmer-line flux from the nebula is easy to measure, the visual magnitude of a hot central star is not. When the central star is not visible on photographs, it is possible to use large-aperture photometry to measure the line and continuum strengths of the nebula and, by applying a theoretical correction to the strength of the nebular continuum, to estimate the contribution to the visual continuum from the star^{4,5}. Such a technique is difficult to apply to stars fainter than about $m_v = 14$ because of the noise from the background night sky and the nebula. A more sensitive search technique^{6,7} is to image the nebula using narrow-band filters to exclude all of the nebular emission lines, thus ensuring maximum contrast between the star (which is mainly a continuum source) and the nebula (the spectrum of which is dominated by emission lines).

NGC2440 is a fairly bright nebula lying close to the galactic plane. Shaw and Kaler⁸ have derived a lower-limit magnitude of $m_v = 14.3$ for the central star using the technique of large-aperture photometry. Curtis⁹ failed to find the central star on photographs in a survey which went down to $m_v = 19.5$, and Pottasch¹ used this lower limit to estimate the central star temperature as $5 \times 10^5 \text{ K}$. Kohoutek and Martin⁵ have disputed this estimate and claimed the star temperature to be only 10^5 K . Reay *et al.*⁷ have given a lower limit of $m_v = 16$ ($T = 180,000 \text{ K}$).

In order to resolve this controversy we have made new observations with a CCD camera at the prime focus of the Anglo-Australian telescope, using a $10\text{-\AA}$ -wide filter centred at $4,788 \text{ \AA}$. This wavelength was chosen to exclude any known nebular emission lines, and thus to maximize the contrast of the star against the background.

The images we obtained are shown as contour maps in Fig. 1. Figure 1a shows the central star clearly between the two inner condensations. The seeing for this exposure, determined from the profiles of field stars, was less than 1.0 arc s FWHM . Figure 1b shows an observation taken using the same detector-filter combination one day earlier, when the seeing was 2.0 arc s . The absence of the star is explained entirely by the poorer seeing, as indicated by Fig. 1c, which shows Fig. 1a smoothed to the same spatial resolution as Fig. 1b. The central star is simply drowned by the nebular continuum emission when the seeing is poor.

The magnitude of the central star was determined by interpolating and subtracting the nebular continuum from Fig. 1a and integrating under the stellar profile to determine the total flux. Comparison of this with similar quantities determined for two nearby field stars, the magnitudes and spectral types of which have been determined by Gathier¹⁰, enabled the visual magnitude of the central star to be determined by making the assumption that it is sufficiently hot to have a Rayleigh–Jeans black-body-like spectrum in the visible and beyond, and assuming the comparison stars have black-body spectra. An independent determination of the magnitude was made by comparison with a standard star (101–281) of known magnitude and spectral type¹¹. Again it was necessary to assume that both the central star and standard star have black-body-like spectra in the mid-visible. From these independent calibrations we obtain an average value of 18.9 ± 0.2 for the magnitude of the central star.

The hydrogen Zanstra temperature $T_Z(\text{H})$ is determined as follows. Using the calibration of Wamsteker¹², that 0 magnitude in the visual corresponds to $3.4 \times 10^{-9} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ \AA}^{-1}$, the magnitude (18.9) of the star is equivalent to a continuum flux

‡ Present address: Queensgate Instruments Ltd, 112 Windmill Road, Sunbury-on-Thames, TW16 7HB, UK.

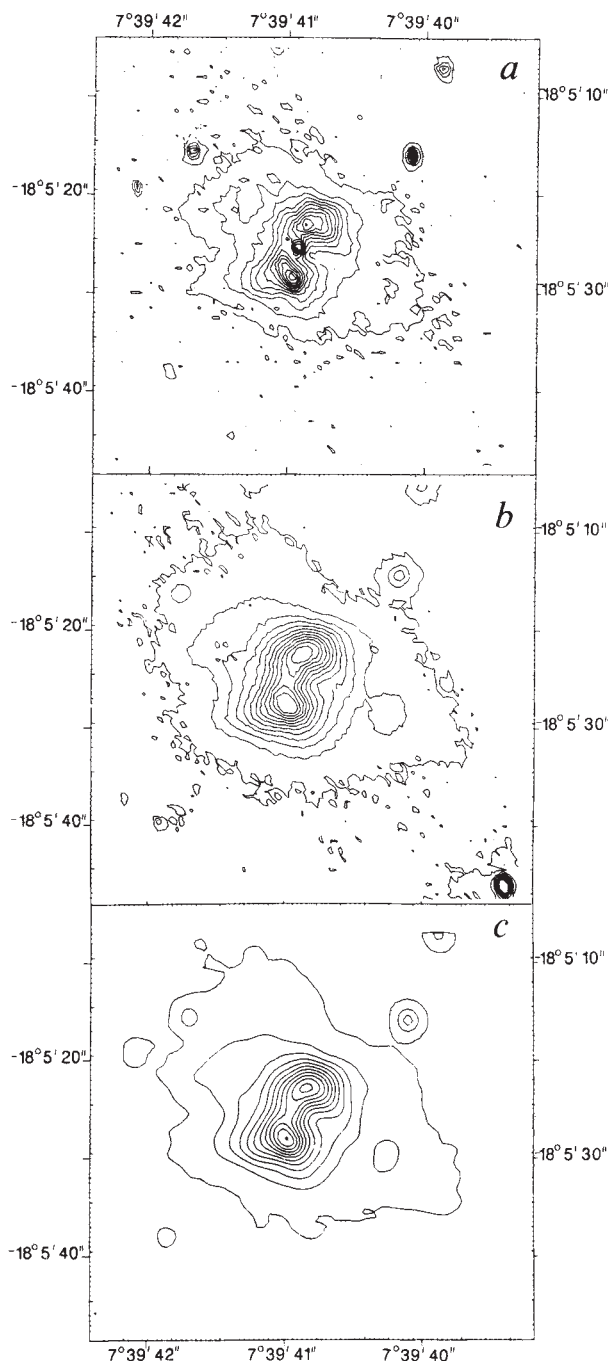


Fig. 1 Narrow-band CCD images of NGC2440 obtained through a continuum filter centred at $\lambda = 4,788 \text{ \AA}$. In *a*, the seeing was 1 arc s, and the central star is clearly visible between the inner pair of condensations. *b*, CCD image recorded on previous night when the seeing was 2 arc s. The star is no longer visible. *c*, Image *a* smoothed to a spatial resolution of 2 arc s. The central star is no longer visible, illustrating the critical importance of seeing conditions to detectability.

of $1.0 \times 10^{-16} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ \AA}^{-1}$. The $H\beta$ flux emitted by the nebula is $3.6 \times 10^{-11} \text{ erg cm}^{-1} \text{ s}^{-1}$ (ref. 4); the ratio of continuum to $H\beta$ flux is thus $2.8 \times 10^{-6} \text{ \AA}^{-1}$ after correction for a reddening of $E_{B-V} = 0.30$ (ref. 10). This ratio corresponds to $T_Z(\text{H}) = 4.0 \times 10^5 \text{ K}$.

The ionized helium Zanstra temperature is computed from the above and the ratio $\text{He II}(4,686 \text{ \AA})/H\beta$ of 0.6 (ref. 13). The ratio of continuum to 4,686- $\text{\AA}$ flux, $4.6 \times 10^{-6} \text{ \AA}^{-1}$, corresponds to a $T_Z(\text{He II})$ of $3.1 \times 10^5 \text{ K}$. Errors in $T_Z(\text{H})$ and $T_Z(\text{He II})$, due mainly to the error of ± 0.2 in the stellar magnitude, amount

to $\pm 25,000 \text{ K}$ and $\pm 20,000 \text{ K}$ respectively. The difference between $T_Z(\text{H})$ and $T_Z(\text{He II})$ is probably due to $T_Z(\text{H})$ being overestimated because recombination from He^{2+} yields more than one photon capable of ionizing hydrogen. On the other hand, $T_Z(\text{He II})$ is an underestimate for the effective temperature, T_{eff} , since some of the photons capable of ionizing He^+ are actually absorbed by H. These effects become more pronounced at higher temperatures¹⁴. A good estimate of the effective temperature is $350,000 \pm 25,000 \text{ K}$. Notably, in ref. 1 the same effect is reported in NGC7027. These consistent results confirm the assumption that the nebula is optically thick for photons ionizing both hydrogen and helium.

The Zanstra temperature may be compared with that determined from the energy balance method. Preite-Martinez and Pottasch¹⁵ find $T = 186,000 \text{ K}$ for the central star of NGC2440, but the temperature determination is quite sensitive to the He^{2+} abundance. If an abundance ratio $N_{\text{He}^{2+}}/N_{\text{H}} = 0.053$ is used, which follows from the measurement of Shields *et al.*¹³, the temperature becomes $T = 230,000 \text{ K}$. This is slightly lower than the Zanstra temperature. If account is taken of the energy emitted by the nebula in the far-infrared and in ultraviolet transitions not seen by the IUE satellite, then the agreement of the two methods is excellent.

The angular radius can now be computed from the visual flux given above. A correction for extinction must be made: we have used $E_{B-V} = 0.30$ (ref. 10). The angular radius is then $R/d = 9.8 \times 10^{-14}$, where R is the stellar radius and d is the distance to the nebula. The value of d is rather well known¹⁰; we used $d = 2.3 \text{ kpc}$. Thus $R = 6.8 \times 10^8 \text{ cm} = 0.99 \times 10^{-2} R_{\odot}$ and the luminosity is about $2,600 L_{\odot}$.

Only one set of evolutionary calculations has been published for stars more massive than $0.7 M_{\odot}$: those of Paczynski¹⁶ for $0.8 M_{\odot}$ and $1.2 M_{\odot}$. More recent calculations of the evolution of stars near $0.6 M_{\odot}$ (ref. 17) confirm the general trends of the earlier models. As shell nuclear burning proceeds in a star, its temperature increases to a maximum when the shell sources have used up essentially all the available hydrogen and helium, and then begins to decrease. The maximum temperature depends critically on the nebular mass. If the models of Paczynski¹⁶ are correct, it can reach the value observed for NGC2440 only when its mass is $\geq 1.0 M_{\odot}$. The high nitrogen and helium abundances observed in NGC2440 (ref. 3) support our contention that the central star has a mass $\geq 1.0 M_{\odot}$. For a $1.0 M_{\odot}$ model, the time remaining until nuclear burning stops is about 300 yr. But the nebula must be considerably older. The age of the nebula can be determined from its diameter and expansion velocity (0.32 pc ; 23 km s^{-1}) to be $\sim 7,000 \text{ yr}$. But with the present luminosity the cooling time is only $\sim 1,000 \text{ yr}$. This discrepancy between the observed and expected age is significant and probably indicates that the theory is incomplete.

Another problem which arises is the following: It is clear that the progenitor of a white dwarf of $1 M_{\odot}$ must be quite massive. A recent discussion¹⁸, based on a study of white dwarfs in open clusters, places this progenitor mass at $7 M_{\odot}$. But the nebular mass in NGC2440 is only $0.4 M_{\odot}$ (ref. 3). Thus $5 M_{\odot}$ must have been ejected in the course of the evolution of the star, but does not appear to be present in the neighbourhood of the star. While the material could have been ejected at a very early stage in the evolution, this appears unlikely because the most active phase of mass loss appears to be the red giant stage and its transition to a planetary nebula. Thus either the material is in some form which is currently undetectable (molecular hydrogen?) or present estimates of the progenitor mass are much too high.

Received 23 September 1985; accepted 2 January 1986.

1. Pottasch, S. R. *Astr. Astrophys.* **94**, L13-L16 (1981).
2. Zanstra, H. *Publs. Dom. astrophys. Obs.* **4**, 209-260 (1931).
3. Pottasch, S. R. *Planetary Nebula* (Reidel, Dordrecht, 1984).
4. Kaler, J. B. *Astrophys. J.* **210**, 113-119 (1976).
5. Kohoutek, L. & Martin, W. *Astr. Astrophys.* **94**, 365-372 (1981).
6. Atherton, P. D. *et al. Astrophys. J.* **232**, 786-796 (1979).

7. Reay, N. K., Pottasch, S. R., Atherton, P. D. & Taylor, K. *Astr. Astrophys.* **137**, 113-116 (1984).
8. Shaw, R. A. & Kaler, J. B. *Astrophys. J.* **295**, 537-546 (1985).
9. Curtis, H. D. *Univ. Calif. Publ. Lick Obs.* **13**, 57-74 (1918).
10. Gathier, R. thesis, Univ. Groningen (1984).
11. Landolt, A. U. *Astr. J.* **88**, 439-460 (1983).
12. Wamsteker, W. *Astr. Astrophys.* **97**, 329-333 (1981).
13. Shields, G. A., Aller, L. H., Keyes, C. D. & Czyzak, S. J. *Astrophys. J.* **248**, 569-583 (1981).
14. Stasinka, G. & Tyndal, R. *Astr. Astrophys.* (submitted).
15. Preite-Martinez, A. & Pottasch, S. R. *Astr. Astrophys.* **126**, 31-44 (1983).
16. Paczynski, B. *Acta astr.* **21**, 417-435 (1971).
17. Schonberger, D. *Astr. Astrophys.* **103**, 118-130 (1981).
18. Weidmann, V. & Koester, D. *Astr. Astrophys.* **121**, 77-84 (1983).

Fossil tracks of α -particle interactions in minerals

P. B. Price & M. H. Salamon

Department of Physics, University of California, Berkeley, California 94720, USA

Fossil tracks of length $\sim 20 \mu\text{m}$ created by the spontaneous fission of ^{238}U impurities were discovered in 1962¹ and form the basis of the well-known fission-track dating technique, which is broadly applicable to minerals and natural glasses². Fossil tracks formed by recoil daughter nuclei released in the α decay of nuclei in the Th and U decay chains were discovered in 1967³, but because of their very short ranges ($< 0.02 \mu\text{m}$) have been seen only on cleavage planes of mica crystals. (Artificially produced α -recoil tracks have also been seen in albite⁴.) During a search for tracks of slow, supermassive magnetic monopoles in large muscovite mica crystals^{5,6}, we have discovered a third type of natural track caused by nuclear interactions of α particles from radioactive impurities with nuclei in mica. These ' α -interaction tracks', with length intermediate between fission tracks and α -recoil tracks, provide a measure of mild thermal events and are valuable in simulating the appearance and stability of tracks of hypothetical monopole-nucleus bound pairs.

Price *et al.*⁷ showed that a supermassive monopole with speed $v \approx 0.001c$ such as is predicted by grand unified theories would produce a detectable radiation-damage track in mica provided

it first attached to itself a nucleus with a large magnetic moment such as ^{27}Al while passing through the Earth's crust. In an analysis of measurements^{8,9} of the response of mica to accelerated ions of ^{27}Al and other species, Price *et al.*⁷ showed that at low velocities where nuclear stopping dominates over electronic stopping, the rate of etching, v_T , along the trajectory of an ion in muscovite mica depends on the nuclear stopping power S_n as:

$$v_T = (0.012 \mu\text{m h}^{-1}) \times (S_n/1 \text{ GeV cm}^2 \text{ g}^{-1}) \quad (1)$$

for mica etched in 40% HF at 25 °C. To calculate S_n for a monopole-nucleus bound pair, one simply replaces the mass of the nucleus by the effectively infinite ($\sim 10^{16}$ AMU) mass of the pair in the semi-empirical expression for S_n (ref. 10).

In our monopole search^{5,6}, we etched for $t = 48 \text{ h}$, a much longer time than that used to reveal fission tracks, which ensured that an etch pit of ~ 0.25 – 1.6 μm depth would be seen on each cleavage surface if the monopole were bound to a ^{27}Al nucleus and travelling with speed 0.0002 – $0.0016c$ at vertical incidence. Our procedure was to cleave and etch several successive mica sheets, each $\sim 100 \mu\text{m}$ thick (several times greater than the range of a fission fragment), re-align the layers for viewing in transmitted light, and scan for shallow etch pits that recurred on all cleavage surfaces in a linear array indicating the trajectory of a deeply penetrating particle. As shown in Fig. 1, we found three populations of etch pit pairs, distinctly different in length, which matched on two adjacent cleavage surfaces: (1) As found previously^{2,11,12}, fission fragment etch pit pairs have a mean combined length $\langle L \rangle \approx 20 \mu\text{m}$ in young, unannealed micas, and a mean length in old micas that is less than $20 \mu\text{m}$ by an amount which depends on the degree of thermal annealing; (2) shallow etch pits with depths $\leq 0.02 \mu\text{m}$ as measured with a Tolansky multiple-beam interference device are easily visible in phase contrast and can be discriminated against in a monopole search by using bright-field illumination; (3) etch pairs with combined lengths of ~ 0.25 – $1.5 \mu\text{m}$ measured with the Tolansky device occur at a frequency ~ 0.03 – 0.3 times the frequency of occurrence of fission tracks. Since the correlation does not continue to adjacent sheets, monopole tracks are ruled out.

The surface density of etch pit pairs of intermediate length is strongly correlated with the densities of fission tracks and α -recoil pits. We denote surface densities of the three types of

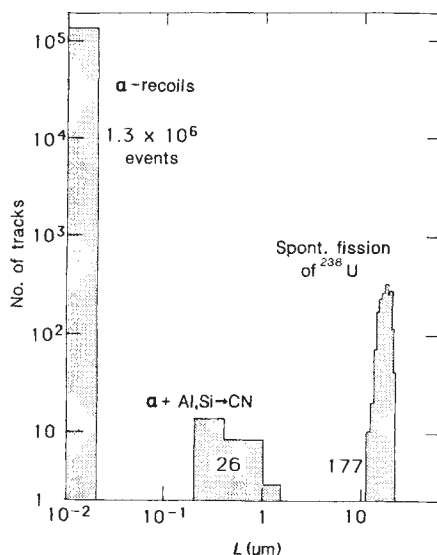


Fig. 1 Distribution of lengths of naturally occurring tracks intersecting an internal cleavage plane in muscovite mica. To measure the track lengths, L , we used an etch time of 4 h in 48% HF at 20 °C instead of the 40-h etch used in our monopole search. The track length distribution consists of three distinct peaks, each of which is labelled with the source of the track-forming particles and the number of events observed. CN, compound nucleus.

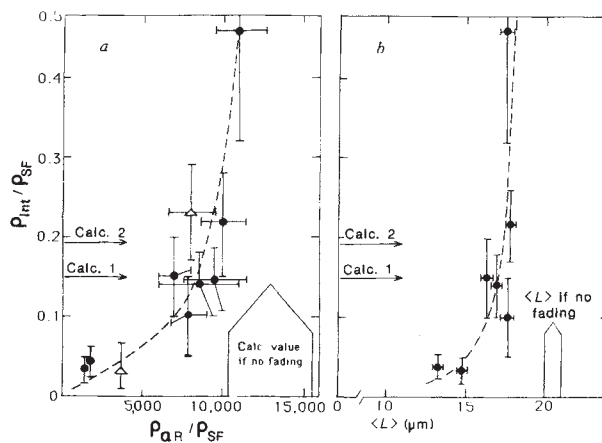


Fig. 2 *a*, Correlation of the ratio $\rho_{\text{int}}/\rho_{\text{SF}}$ with $\rho_{\alpha R}/\rho_{\text{SF}}$ for samples of muscovite with values of ρ_{SF} ranging from 40 to 1,600 tracks per cm^2 and with fission track ages of 400–950 Myr. The values of $\rho_{\text{int}}/\rho_{\text{SF}}$ labelled 'Calc. 1' and 'Calc. 2' on the ordinate are discussed in the text. The calculated value of $\rho_{\alpha R}/\rho_{\text{SF}}$ indicated by an arrow on the abscissa is taken from ref. 3. *b*, Correlation of $\rho_{\text{int}}/\rho_{\text{SF}}$ with mean combined fission track length ($\langle L \rangle$) for various samples of muscovite.

track in number per cm^2 by ρ_{int} , ρ_{SF} and $\rho_{\alpha\text{R}}$ for intermediate, spontaneous fission and α -recoil respectively. Figure 2a shows the strong correlation of the ratio $\rho_{\text{int}}/\rho_{\text{SF}}$ with $\rho_{\alpha\text{R}}/\rho_{\text{SF}}$ in large muscovite crystals selected for our monopole search, and in two crystals that we annealed for 1 h at 300 °C and 250 °C (triangles). Figure 2b shows the correlation of $\rho_{\text{int}}/\rho_{\text{SF}}$ with mean fission track length $\langle L \rangle$ in the same samples. We note that, despite a factor of 40 variation in ρ_{SF} among these micas, ρ_{int} correlates closely with ρ_{SF} and with $\rho_{\alpha\text{R}}$ in such a way as to suggest strongly that these tracks of intermediate length are related to radiation from either U or Th, that they are much less resistant to thermal annealing than are fission tracks, and that they are somewhat less resistant to thermal annealing than are α -recoil tracks. The small ratio $\rho_{\text{int}}/\rho_{\text{SF}}$ in micas with small $\rho_{\alpha\text{R}}/\rho_{\text{SF}}$ and $\langle L \rangle$ thus seems to be due to partial annealing. Table 1 compares the

Table 1 Natural radiation tracks in mica compared with hypothetical tracks of monopole-nucleus bound pairs

Track type	Z	β	dE/dx (GeV cm^2 g^{-1})	R (μm)*
Spontaneous fission	~ 38 and ~ 52	~ 0.031 – 0.046	~ 25	~ 20
α Recoil	82–90	0.0013	~ 15	0.01–0.03
α Interaction	14–15	0.006	~ 3	0.25–1.6
^{27}Al -monopole	13	~ 0.001	~ 3	$>10^{11}\dagger$

Z, atomic number; β , velocity (units of c); dE/dx , rate of energy loss along the track.

* Sum of ranges of both fragments.

† Although the range is $>10^2$ km, as a consequence of the low v_T the etch pits are shallow at every surface traversed.

characteristics of the three types of natural radiation tracks of hypothetical tracks of ^{27}Al -monopole bound pairs.

We have investigated two candidates for the origin of the etch pits of intermediate length. The first is the recently discovered¹³ spontaneous emission of heavy ions by one or more daughter nuclei in the U or Th decay chain. Such an explanation would be viable only if the ratio of decay rates for heavy ion emission relative to spontaneous fission were related to the observed ratios $\rho_{\text{int}}/\rho_{\text{SF}}$ and to the ratios of etchable range of daughter nucleus following heavy ion emission to fission fragment range $\langle L \rangle$ as follows:

$$\rho_{\text{int}}/\rho_{\text{SF}} = (\sum \lambda_i C_i R_i) / \lambda_{\text{SF}} C_{238} \langle L \rangle \quad (2)$$

where λ_i is the decay rate for emission of a particular heavy ion from a nuclide with concentration C_i and etchable range R_i , and λ_{SF} and C_{238} are the spontaneous fission rate and concentration of ^{238}U . Typically, $R_i \approx 2$ – $3 \mu\text{m}$ in mica (only the recoiling daughter, not the energetic heavy ion, has a high enough radiation-damage rate to contribute) and $\rho_{\text{int}}/\rho_{\text{SF}}$ is observed to be ~ 0.1 , which would require $(\sum \lambda_i C_i) / \lambda_{\text{SF}} C_{238} \approx 1$ or $(\sum \lambda_i C_i) / \lambda_{\alpha} C_U \approx \sum B(\text{HI}/\alpha) \approx 10^{-6}$, where $B(\text{HI}/\alpha)$ is the branching ratio for a particular heavy-ion emission mode relative to α -decay. None of the measured¹⁴ or theoretically predicted¹⁵ values of $B(\text{HI}/\alpha)$ for radioactive heavy nuclides is as high as 10^{-9} . It seems very unlikely that more than $\sim 10^{-3}$ of the etch pits of intermediate length could be due to spontaneous heavy-ion emission.

The second explanation, which we believe to be the correct one, is that the etch pits of intermediate length are produced by recoiling compound nuclei resulting from (α, n) and (α, p) reactions of α particles (from the radioactive decay of ^{232}Th , ^{235}U and ^{238}U and their daughters) with nuclei comprising the mica structure.

Crozaz *et al.*¹⁶ showed by accelerator calibrations that energetic He ions from solar flares could produce short 'interaction tracks' in lunar and meteoritic samples and cosmic dust grains, but did not realize that such tracks could also be produced as

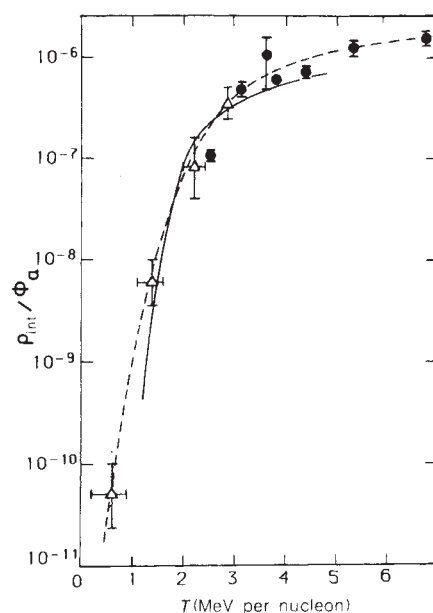


Fig. 3 Production rate of α -interaction tracks per incident He ion as a function of He ion kinetic energy (T). Experimental points are from ref. 16 ($\bullet$) and this work (Δ). The dashed line is an empirical fit to the data; the solid line is calculated from the cross-sections for (α, p) and (α, n) reactions with Al and Si (equation (3)).

a by-product of α decay in terrestrial minerals. Figure 3 shows measurements of the ratio of the density of shallow etch pit pairs on an internal cleavage plane of mica to Φ_{α} , the number of He ions per cm^2 , as a function of He ion kinetic energy (T). The solid points were obtained by Crozaz *et al.*¹⁶, who slowed a 30-MeV He-ion beam to various energies with absorbers; the open circles are data we obtained at the Lawrence Berkeley Laboratory 88-inch cyclotron using He-ion beams of 11.6 and 14 MeV to bombard partially cleaved mica samples with cleavage planes at depths of ~ 20 – $60 \mu\text{m}$. (We annealed the samples for 5 h at 490 °C in air to erase all natural tracks before doing the irradiations.) We calculated the He ion energies at the cleavage planes using a range-energy relation for He ions in muscovite¹⁹.

We found that, in addition to etch pairs with lengths of ~ 0.25 – $1.6 \mu\text{m}$, very faint etch pits, visible only in phase contrast, result from He ion bombardments. Their production rate does not show a threshold at low energies. It is likely that they are simply atoms scattered elastically by the He ions and that their small V_T is due to their having a smaller charge than the recoiling compound nuclei.

In their study, Crozaz *et al.*¹⁶ found that (1) He ions produced 'interaction tracks' in several minerals in addition to muscovite; (2) the ratio $\rho_{\text{int}}/\Phi_{\alpha}$ increased rapidly above a threshold at ~ 2 MeV per nucleon (see Fig. 3); (3) $\rho_{\text{int}}/\Phi_{\alpha}$ was four times higher if the He ions passed through an Al cover foil rather than through a muscovite cover; (4) $\rho_{\text{int}}/\Phi_{\alpha}$ was >100 times lower for a plastic cover (containing H, C and O nuclei) than for a muscovite cover; (5) the tracks could be annealed out at a much lower temperature than fission tracks. They inferred from the energy threshold that α -induced nuclear reactions are a more important source than elastically scattered nuclei.

Since the work of Crozaz *et al.*¹⁶, cross-sections as a function of energy for reactions of He ions with Al and Si targets have been measured¹⁷, and data on the registration of tracks as a function of Z and velocity in mica have been obtained (refs 8, 9, 18 and P.B.P. and M.H.S., unpublished data). From these

data we have been able to calculate the production rate $\rho_{\text{int}}/\Phi_{\alpha}$ as a function of energy, shown by the solid curve in Fig. 3, based on the following expression:

$$\rho_{\text{int}}/\Phi_{\alpha} = \sum_j C\sigma_j(E)r_j(E) \quad (3)$$

where $C = C_{\text{Al}} = C_{\text{Si}} = 1.28 \times 10^{22} \text{ cm}^{-3}$ is the number of Al or Si nuclei per cm^3 in muscovite of composition $\text{K}_2\text{Al}_4[\text{Si}_6\text{Al}_2\text{O}_{20}](\text{OH},\text{F})_4$; $\sigma_j(E)$ is the cross-section for a particular (α, p) or (α, n) reaction on Al or Si; $r_j(E)$ is the recordable range of recoiling residual nuclei following compound nucleus de-excitation; the sum is over both types of reactions and both types of nuclei. Since values of r_j are typically $< 1 \mu\text{m}$, which is much smaller than the range of the α particles in the beam, the relevant kinetic energy E can be taken as constant and equal to that of He ions at the cleavage plane. Equation (3) assumes a one-dimensional geometry, which is approximately valid for recoiling compound nuclei emitting a neutron or proton.

Because of their very low etching rate, v_T , we have ignored contributions of products of He-induced reactions with O and F; in view of the low concentration of K relative to Al + Si, and in the absence of cross-section data, we have ignored He-induced reactions with K. The agreement between the calculated curve and the two sets of data is satisfactory when uncertainties in He-ion energies at the mica cleavage plane and in values of r_j are taken into account. Therefore, following Crozaz *et al.*¹⁶, we refer to the etch-pit pairs of intermediate length as α -interaction tracks.

Table 2 shows the contributions of four of the α -emitting nuclei in the U and Th decay chains to the concentration of α -interaction tracks relative to the concentration of spontaneous fission tracks. α Particles from other nuclides have too low an energy or, in the case of the ^{235}U chain, too low an abundance to contribute significantly. We used two methods to calculate the ratio $\rho_{\text{int}}/\rho_{\text{SF}}$.

The first approach was, for a concentration C_i and decay rate λ_i of an α -emitter of species i , to calculate the flux of α particles in each interval of residual range seen by Al and Si nuclei located close enough to a cleavage plane to produce α -interaction tracks crossing that cleavage plane from either side. In the integral in equation (4), the cross-section σ_p , the recordable range r_j of the recoiling product of the compound-nucleus reaction of the α -particle with Al or Si, and the residual range R_i of an α particle from species i are functions of the residual kinetic energy of the slowing α particle.

$$\left(\frac{\rho_{\text{int}}}{\rho_{\text{SF}}}\right)_i = \frac{\lambda_i C_i}{\lambda_{\text{SF}} C_{238}} \frac{2C \sum_j \int_0^{R_{\text{max},i}} \sigma_j r_j dR_i}{\langle L \rangle} \quad (4)$$

The sum is over (α, p) and (α, n) reactions with Al and Si. The expression for the ratio $(\rho_{\text{int}}/\rho_{\text{SF}})_i$ has implicitly taken into account an integration over isotropic distributions of directions of α emission and spontaneous fission, which leads to mean thicknesses $R_i/2$ for α emission and $\langle L \rangle/2$ for fission. Based on calibration data, we take r_j to be the true range of the recoil minus $0.15 \mu\text{m}$, the portion at the end of the range with v_T too small to be detected. In secular equilibrium the ratio of activities per cm^3 , $\lambda_i C_i/\lambda_{\text{SF}} C_{238}$, is taken to be 2.8×10^6 for all members of the ^{232}Th decay chain and 2.2×10^6 for members of the ^{238}U chain. The results are shown in the penultimate column of Table

Table 2 Production of α -interaction tracks by U, Th decay chains

Parent	Daughter	T_{α} (MeV)	Calc. 1	Calc. 2
^{232}Th	^{212}Po	8.79	0.13	0.13
^{232}Th	^{216}Po	6.84	0.009	0.018
^{238}U	^{214}Po	7.69	0.008	0.036
^{232}Th	^{220}Rn	6.29	0.003	0.009
Total from all sources			0.15	0.193

2. The sum of the contributions from the four species gives $\rho_{\text{int}}/\rho_{\text{SF}} = 0.15$, which is indicated on the ordinates of Fig. 2 by an arrow labelled 'Calc. 1'.

The second approach was to use the He-ion calibration data for $\rho_{\text{int}}/\Phi_{\alpha}$ as a function of energy (Fig. 3) to calculate $(\rho_{\text{int}}/\rho_{\text{SF}})_i$ for each species i :

$$\left(\frac{\rho_{\text{int}}}{\rho_{\text{SF}}}\right)_i = \frac{\lambda_i C_i}{\lambda_{\text{SF}} C_{238}} \frac{\int_0^{R_{\text{max},i}} (\rho_{\text{int}}/\Phi_{\alpha}) dR_i}{\langle L \rangle} \quad (5)$$

The results are shown in the last column of Table 2 and by the arrow labelled 'Calc. 2' on the ordinates in Fig. 2.

The strong correlation of the ratio $\rho_{\text{int}}/\rho_{\text{SF}}$ with $\rho_{\alpha\text{R}}/\rho_{\text{SF}}$ and $\langle L \rangle$ for micas varying by a factor of 40 in U concentration, the agreement in length distribution of natural etch pit pairs of intermediate length and of those produced in He-ion irradiations, and the close agreement between the values of $\rho_{\text{int}}/\rho_{\text{SF}}$ obtained from the two calculations and the value obtained in the majority of the measurements shown in Fig. 2 establish that the naturally occurring etch pit pairs of intermediate length are due to recoil compound-nuclei produced in reactions of α particles emitted by daughters of U and Th impurities in mica.

Resistance to thermal fading has been shown² to depend on radiation damage density associated with a particular type of track. Based on the relative energy deposition rates shown in Table 2, we would expect α -interaction tracks to be less resistant to thermal fading than α -recoil tracks, which in turn would be less resistant than fission tracks. This is borne out by the correlations of $\rho_{\text{int}}/\rho_{\text{SF}}$ with $\rho_{\alpha\text{R}}/\rho_{\text{SF}}$ and $\langle L \rangle$, which we have indicated by dashed lines in Fig. 2. All of the micas for which data are presented in Fig. 2 were selected on the basis of their perfection, large size and great age for use in our monopole search. Because of the close similarity in radiation-damage rate for an Al-monopole track and for an α -interaction track (Table 1), we can use the observed ratio $\rho_{\text{int}}/\rho_{\text{SF}}$ as a measure of survivability of Al-monopole tracks. We conclude that all but the two samples with $\rho_{\text{int}}/\rho_{\text{SF}} < 0.1$ would have retained Al-monopole tracks during most if not all of the timespan indicated by their fission track ages.

This work was supported in part by NSF grant PHY-8403710. We thank Dr A. M. Clark (British Museum of Natural History), Dr B. Mason (US National Museum) and Professor Gordon Brown (Stanford University) for supplying mica samples, Ruth-Mary Larimer for assistance with the He-ion irradiations, Dr C. E. Naeser for assistance with fission-track ages, Professor M. Majda for the use of his Tolansky interference device, Dr Eric Norman for a discussion and Michael Solarz for assistance with the etching.

Received 28 October 1985; accepted 6 February 1986.

- Price, P. B. & Walker, R. M. *Nature* **16**, 732-734 (1962).
- Fleischer, R. L., Price, P. B. & Walker, R. M. *Nuclear Tracks in Solids: Principles and Applications* (University of California Press, Berkeley, 1975).
- Huang, W. H. & Walker, R. M. *Science* **155**, 1103-1106 (1967).
- Turkowsky, C. *Earth planet. Sci. Lett.* **5**, 492-496 (1969).
- Price, P. B. & Salamon, M. H. *Proc. 19th Int. Cosmic Ray Conf., La Jolla* **8**, 242-245 (National Aeronautics and Space Administration, Washington, D.C., 1985).
- Price, P. B. & Salamon, M. H. *Phys. Rev. Lett.* (in the press).
- Price, P. B., Guo, S.-L., Ahlen, S. P. & Fleischer, R. L. *Phys. Rev. Lett.* **52**, 1265-1268 (1984).
- Borg, J., Dran, J. C., Langevin, Y., Maurette, M. & Petit, J. C. *Radiat. Effects* **65**, 373-377 (1982).
- Borg, J. thesis, Univ. Paris (1980).

- Wilson, W. D., Haggmark, L. G. & Biersack, J. P. *Phys. Rev.* **B15**, 2458-2468 (1977).
- Bigazzi, G. *Earth planet. Sci. Lett.* **3**, 434-438 (1967).
- Mehta, P. P. & Rama *Earth planet. Sci. Lett.* **7**, 82-86 (1969).
- Rose, H. J. & Jones, G. A. *Nature* **307**, 245-247 (1984).
- Price, P. B. *Phys. Bull.* **36**, 489-490 (1985).
- Poenaru, D., Ivascu, M., Sandulescu, A. & Greiner, W. *Phys. Rev.* **C32**, 572-581 (1985).
- Crozaz, G., Hair, M., Maurette, M. & Walker, R. *Proc. int. top. Conf. Nuclear Track Registration in Insulating Solids and Applications*, Clermont-Ferrand Vol. 2, 41-54 (Laboratoire de Physique Nucléaire, Clermont-Ferrand, 1969).
- Seamster, A. G., Norman, E. B., Leach, D. D., Dyer, P. & Bodansky, D. *Phys. Rev.* **C29**, 394-402 (1984).
- Bibring, J. *et al. Proc. 8th int. Conf. Nuclear Photography and Solid State Track Detectors*, Bucharest Vol. 1, 485-499 (Institute of Atomic Physics, Bucharest, 1972).
- Salamon, M. H. *Lawrence Berkeley Lab. Rep.* No. 10446 (1980).

Incommensurately modulated α' - Sr_2SiO_4

L. Stenberg*, J. R. Sellar* & B. G. Hyde†

Research School of Chemistry, The Australian National University,
GPO Box 4, Canberra, ACT 2601, Australia

The solid-state chemistry of superficially mundane A_2BX_4 compounds with $\beta\text{-K}_2\text{SO}_4$ -related structures is 'a can of worms', characterized by prolific polymorphism and, despite careful single-crystal X-ray diffraction studies, persistent uncertainty about exact space groups and atom positions. This is true of Ca_2SiO_4 ¹⁻⁷, a major constituent of cement, and Sr_2SiO_4 ⁸⁻¹⁰ despite 50 years of (confusing) literature. On the other hand, the solid-state physics of some other A_2BX_4 compounds (for example, K_2SeO_4), as well as more exotic charge-density-wave types, involves modulated structures. Similar behaviour could conceivably resolve the structural confusion cited above (wrong structural models may have been refined); to investigate this possibility¹¹ we have studied the transition $\beta \rightarrow \alpha'$ - Sr_2SiO_4 by beam-heating the β -polymorph in an electron microscope. Electron diffraction patterns reveal two sets of incommensurate satellite reflections, indicating distinct modulated structures. Their origin is an asymmetric —Sr—O—Sr—O— chain (common to β and α'), with alternating bond lengths which tend to equality as T increases, but at a cost of reduced total bond strength and energy.

Although the β structures of both Ca_2SiO_4 and Sr_2SiO_4 are well characterized, our understanding of the α' -structure, and hence of the $\beta \rightleftharpoons \alpha'$ transition, is incomplete. A good refinement of α' - Ca_2SiO_4 is not available, and there has been conjecture about its structure. It now seems to be generally agreed that there are two α' -Ca structures: α'_L at lower and α'_H at higher temperatures^{5,6}. Both are superstructures of a prototype α' ($\beta\text{-K}_2\text{SO}_4$ type, $Pmnb$, $Z=4$); but their nature is subject to dispute. For α'_L some authors deduce $2a \times b \times 2c$ ^{6,12}, others $a \times 3b \times c$ (ref. 3). On the other hand, the recent, careful single-crystal X-ray study of α' - Sr_2SiO_4 (ref. 9) at 110 °C (~ 25 °C above transition temperature, T_{tr}) made no mention of a superstructure (although one would anticipate that Ca_2SiO_4 and Sr_2SiO_4 would be similar). The data were analysed in terms of two basic models: (1) an ('ordered') orthorhombic ($Pmnb$) structure with Sr atoms and SiO_4 tetrahedra on (100) mirror planes; and (2) a ('disordered') monoclinic ($P2_1/n$, but with $\beta = 90^\circ$) twinned structure with these atoms statistically distributed between two sets of close, (100)-mirror-related positions. Model (2) was preferred:

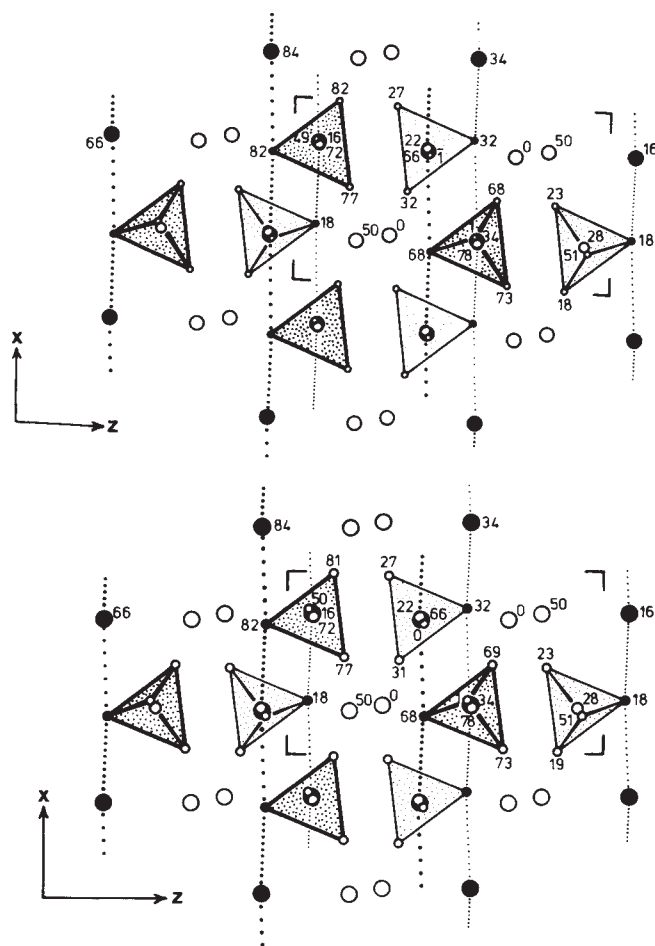


Fig. 2 The structures of $\beta\text{-Sr}_2\text{SiO}_4$ (ref. 8) (top) and the 'disordered' model of $\alpha'\text{-Sr}_2\text{SiO}_4$ (ref. 9) (bottom) projected on (010). Large circles, Sr; medium circles, Si; small circles, O atoms. SiO_4 tetrahedra are shown; dotted lines represent —Sr(1)—O(2)— chains, with the dense dots indicating the shorter bond. (The second twin of each is obtained by reflection in (100).)

it strongly resembles the $\beta\text{-Sr}_2\text{SiO}_4$ structure ($P2_1/n$, $\beta = 92.67^\circ$), crystals of which are always polysynthetically twinned; and $\alpha'\text{-Sr}_2\text{SiO}_4$ is the para-elastic form of the ferroelastic β . Nevertheless, ambiguity remains.

It is conceivable that an incommensurately modulated structure intrudes into the (displacive) $\beta \rightleftharpoons \alpha'$ transition and that electron diffraction studies could reveal this¹¹. The transition

* Permanent addresses: Kemicentrum, Oorganisk Kemi 2, Lunds Universitet, Lund 522100, Sweden (L.S.); and ICI Australia Operations PTY Ltd, Research Group, Newsom Street, Ascotvale, Victoria 3032, Australia (J.R.S.).

† To whom correspondence should be addressed.

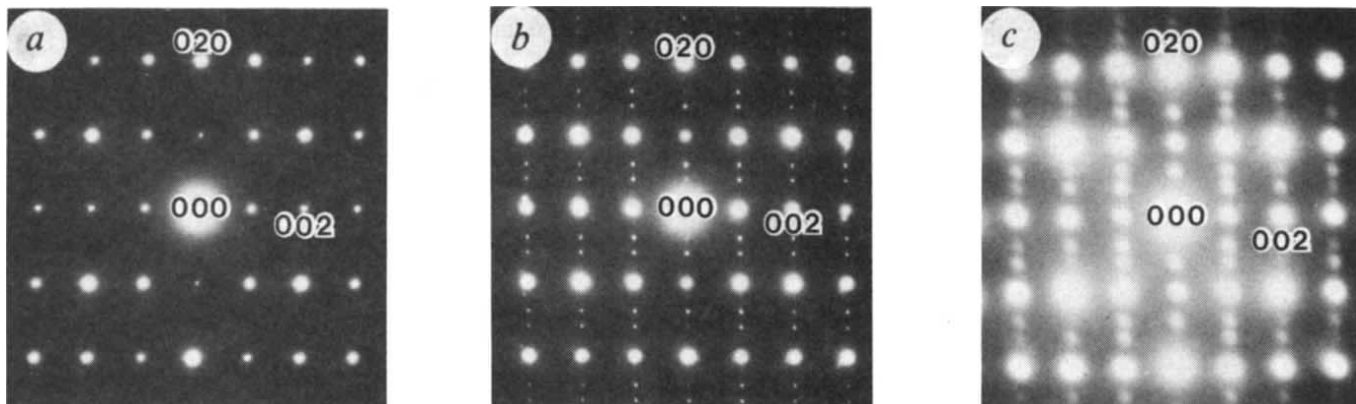


Fig. 1 [100] zone-axis diffraction patterns of a , $\beta\text{-Sr}_2\text{SiO}_4$ (b -axis unique); b , c , beam-heated Sr_2SiO_4 . The latter two show Bragg reflections similar to those in a , but also additional satellite reflections, q_1 in b and q_2 in c .

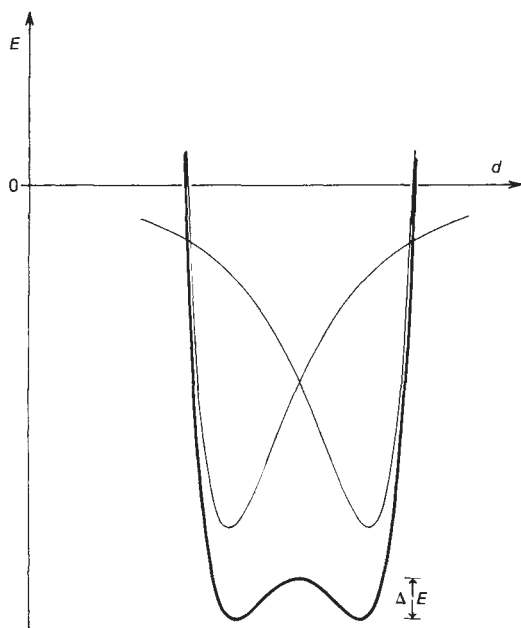


Fig. 3 A schematic representation of the Sr(1)—O(2) bond (potential) energy, E , as a function of bond length, d , in the —Sr(1)—O(2)— chains parallel to x . The thin lines are for adjacent bonds; the solid line is their sum.

temperature is $\sim 680^\circ\text{C}$ for Ca_2SiO_4 (refs 2, 5, 6) but $\sim 85^\circ\text{C}$ (ref. 13) for Sr_2SiO_4 . The lower temperature is more readily accessible in the electron microscope; we have therefore 'beam-heated' Sr_2SiO_4 , because a hot stage was not available. An additional advantage of Sr_2SiO_4 is that it has no γ -polymorph, so that 'dusting', due to $\beta \rightarrow \gamma$ - Ca_2SiO_4 , does not occur. (A previous electron-microscope study of $\beta \rightleftharpoons \alpha'$ - Ca_2SiO_4 has been reported¹⁴, but although micrographs were discussed, diffraction patterns were not.)

Three types of [100] zone-axis diffraction patterns were observed (see Fig. 1). The pattern in a was obtained with a defocussed beam, and corresponds to the β -phase; for b and c a focused (heating) beam was used. In addition to the Bragg reflections observed in a , patterns b and c also contain satellite reflections, the spacing of which is different in the two cases, but apparently incommensurate with the Bragg reflections in both. (b , $q_1 = 0.39b^*$; c , $q_2 = 0.30b^*$.) They correspond to modulations with periodicities of $\sim 2.56b \approx 14.5 \text{ \AA}$ and $\sim 3.33b \approx 18.9 \text{ \AA}$, respectively. Within the error of measurement, no other q values have been detected.

The observations are interesting in at least two respects: (1) the multiplicities (2.56 and 3.33) are close to the value of $3b^*$ reported for an α'_L superstructure by Saalfeld³; (2) the presence of a modulation could well account for the elusive details of the α' -structure referred to above. While the (relatively weak) satellites are clearly consistent with the single-crystal X-ray diffraction data from α' - Sr_2SiO_4 (refs 9, 10), the possibility of modulation appears not to have been considered previously; which is doubly strange as its likelihood was suggested some time ago¹⁵, although not specifically for alkaline earth silicates. Indeed, the transformations $Pmnb \rightleftharpoons \text{incommensurate} \rightleftharpoons P2_1/n$ have recently been reported for some other compounds with β - K_2SO_4 -related structures: Cs_2CdBr_4 and Cs_2HgBr_4 (ref. 16).

The driving force for this transformation (and related ones) is complex, but its major component is simple and direct. Figure 2 shows (one twin form of each of) the β - and α' - Sr_2SiO_4 structures^{8,9} projected along [010], the pseudo-hexagonal (and unique monoclinic) axis (setting $P2_1/n1$) and the modulation direction; in both there are nearly rectilinear chains, —Sr(1)—O(2)—Sr(1)—O(2)—, parallel to x . In these chains, alternate bonds are of different lengths: $l(\text{Sr—O}) = 2.56_\alpha$ (denoting uncertainty, $2.566 \pm 0.00n$) and 3.10_β and 2.68_α and

$3.00_\beta \text{ \AA}$ in α' ; the difference is more marked in β than in α' . This difference is the principal cause of the well known tilting of the SiO_4 tetrahedra from a more asymmetrical position in the β -structure to a more symmetrical one in the α' (α'_L ?) structure and eventually, at even higher temperatures, to a completely symmetrical one and space group $Pmnb$ in the β - K_2SO_4 -type (α'_H ?) structure.

Bond strength calculations¹⁷ yield, for these bonds, $s = 0.28$ and 0.07 valence units for β and 0.21 and 0.10 for α' ; so that the sum of the two bond strengths is 0.35 for β and 0.31 for α' . Clearly the bond energy is higher (and the crystal energy lower) in the former. Equally clearly (and this is emphasized by the invariable polysynthetic twinning of β), the Sr—O bond energy is a 'double-well' function (Fig. 3). With the difference that the chain is diatomic rather than monatomic, this is the simple situation described by Pynn¹⁸ for incommensurate structures. The modulation can be described as a variation, in the b direction, of the relative lengths of the two critical Sr(1)—O(2) bonds (which are almost parallel to a). As a consequence, the tilt of the SiO_4 tetrahedra (about an axis sub-parallel to b , and passing through the Si atoms) varies gradually, and periodically, between two extremes: that in Fig. 2b and its (100)-mirror-related twin.

Similar considerations will almost certainly apply to the analogous calcium silicate, which is now being studied.

Received 25 November 1985; accepted 13 February 1986.

- Smith, D. K., Majumdar, A. & Ordway, F. *Acta crystallogr.* **18**, 787–795 (1965).
- Eysel, W. & Hahn, T. *Z. Kristallogr.* **131**, 322–341 (1970).
- Saalfeld, H. *Am. Miner.* **60**, 824–827 (1975).
- Moore, P. B. & Araki, T. *Am. Miner.* **61**, 74–87 (1976).
- Barnes, P., Fentiman, C. H. & Jeffrey, J. W. *Acta crystallogr.* **A36**, 353–356 (1980).
- Sarkar, S. L. *J. Mater. Sci.* **15**, 1324–1325 (1980).
- Eysel, W., Höfer, H. H., Keester, K. L. & Hahn, T. *Acta crystallogr.* **B41**, 5–11 (1985).
- Catti, M., Gazzoni, G. & Ivaldi, G. *Acta crystallogr.* **C39**, 29–34 (1983).
- Catti, M., Gazzoni, G., Ivaldi, G. & Zanini, G. *Acta crystallogr.* **B39**, 674–679 (1983).
- Catti, M. & Ivaldi, G. *Acta crystallogr.* **B40**, 537–544 (1984).
- Barbier, J. & Hyde, B. G. *Acta crystallogr.* **B41**, 383–390 (1985).
- Regourd, M., Bigare, M., Forest, J. & Guinier, A. *5th int. Symp. Chemistry of Cement*, Tokyo, Vol. 1, 44–48 (The Cement Association of Japan, 1968).
- Catti, M. & Gazzoni, G. *Acta crystallogr.* **B39**, 679–684 (1983).
- Groves, G. W. *J. Mater. Sci.* **18**, 1615–1624 (1983).
- Iizumi, M., Axe, J. D., Shirane, G. & Shimaoka, K. *Phys. Rev.* **B15**, 4392–4411 (1977).
- Altermatt, D., Arend, H., Gramlich, V., Niggli, A. & Petter, W. *Acta crystallogr.* **B40**, 347–350 (1984).
- Brown, I. D. in *Structure and Bonding in Crystals* Vol. 2 (eds O'Keeffe, M. & Navrotsky, A.) (Academic, New York, 1981).
- Pynn, R. *Nature* **281**, 433–437 (1979).

Formation of fractal cracks in a kinetic fracture model

Yves Termonia & Paul Meakin

Central Research and Development Department, Experimental Station, E.I. du Pont de Nemours and Company, Inc., Wilmington, Delaware 19898, USA

Interest is growing in the wide variety of fractal objects¹ found in nature; these include electric discharge patterns², infinite percolation clusters^{3,4}, branched polymers^{5,6}, and surface irregularities⁷. The question of whether surface cracks, such as those that occur in protective coatings, also lead to fractal-like structures is another problem of great interest. Here we study this question theoretically, making use of a molecular model for material failure, introduced previously⁸, which is based on the kinetic theory of fracture^{9,10}. The model is applied to a two-dimensional surface stretched in one direction, and our results show that the cracks developing in the material exhibit properties similar to each other, at least at the molecular level. For a wide range of values of the elastic constants of the material, the fractal dimensionality is found to have a universal value, $D = 1.27 \pm 0.02$.

The surface is represented by a two-dimensional (x - y) network of nodes representing the difficult-to-deform strong parts of the material (Fig. 1). These nodes are joined in the y

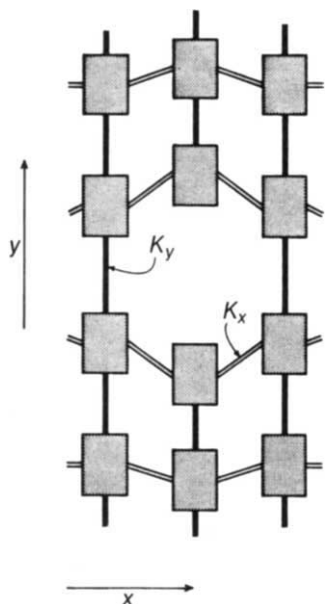


Fig. 1 Two-dimensional network of nodes with one broken bond along the y -axis. K_y and K_x are the elastic constants for the bonds.

and x directions by elastic bonds with force constants K_y and K_x , representing the easily deformable and easily broken regions. In the case of a fibre that is perfectly ordered and oriented along the y -axis, the nodes represent the elementary repetition units of the macromolecules, and K_y and K_x are the elastic constants for the primary (for example, C-C) and secondary (van der Waals or hydrogen) bonds, respectively. The network is stretched along the y -axis by a constant strain and the basic events in the network are controlled by thermally activated bond breakages; accumulation of these events leads to crack formation and, ultimately, to network breakdown. The bond breakage process is examined with the help of the following stochastic model. The (unbroken) bonds are subjected at random to a Monte-Carlo lottery which breaks a bond with probability

$$p_i = \exp \{(-U_i + E_i)/kT\} / p_{\max} \quad (1)$$

where U_i is an activation energy, E_i is the elastic energy stored in the bond, k is Boltzmann's constant, T is the absolute temperature and $p_{\max}$ is a normalization constant which ensures that the probability of breaking the most stressed bond in the array is unity. We set

$$E_i = (1/2)K_i(\Delta l_i)^2 \quad (2)$$

in which K_i is the force constant for bond i and Δl_i is the elongation. As a bond breaks, the two nodes connected through it will move apart (see below). For simplicity, we assume that the motions of the nodes along the x - and y -axes are mutually independent¹⁰, and we focus on their displacements along the y -axis, where the network is strained. Thus, the Δl_i values (see

equation (2)) are for elongations along the y -axis and correspond either to an axial tensile force (for a bond along the y -axis) or to a shear force (for a bond along the x -axis).

After a very small number of bonds have been broken, the network is relaxed to its minimum energy configuration in a series of steps. At each step, a node i is visited and its y -ordinate is adjusted so as to reduce to zero the net residual force (R_i)

$$R_i = \sum_{\text{[intact bonds } j]} K_j(\Delta l_j) + \sum_{\text{[nodes } i' \text{ in contact]}} R_{i'} \quad (3)$$

acting on that node. The first term on the right-hand side of equation (3) is due to the elastic force acting on the bonds j originating from node i , with $K_j = K_y$ and $K_j = K_x$ for a bond along the y - and the x -axis, respectively. Δl_j is the elongation of bond j , measured by the difference in the y -ordinates of the two ends of the bond. The second term on the right-hand side of equation (3) accounts for the external forces acting on node i , when in contact with any of its two neighbours i' along the y -axis. Indeed, we recall our assumption that the nodes are the difficult-to-deform, strong parts of the material.

It is important to note that if the residual force for a node is reduced to zero, it often results in an automatic increase of (the absolute value of) the residuals for the neighbouring nodes. Therefore, the liquidation of all the residuals in the array is a very computer-time-consuming process. For that reason, the liquidation of the residuals has been performed using two well-known computational devices¹¹, called overrelaxation and block relaxation, which considerably speed up the convergence of the calculations. Overrelaxation moves a node not simply to the position at which the residual R is zero, but rather to a position at which the sign of the residual is opposite to that of the average for the neighbouring nodes. The second, and more important, device is known as block relaxation and consists of adjusting the y -ordinates of more than one node at a time.

We start with an intact network (no broken bonds), stretched by a constant strain of 20%. The stochastic model then proceeds in a series of steps. At each step, 0.1% of the total number of bonds are broken according to equations (1) and (2), after which the network is relaxed towards mechanical equilibrium, using equation (3). These steps are repeated until the network breaks. Typically, a network consists of 60 nodes in the y -direction and 100 nodes in the x -direction, with periodic boundary conditions. The small size of the network is dictated by the mechanical relaxation steps which represent the most computer-time-consuming processes in our simulations. A relaxation step was considered to be complete when the net forces measured at the two ends of the network along the y -axis were equal to within a few per cent. A typical simulation takes 6–8 hours of CPU time on an IBM 3081D. For the calculation of the elongations Δl_i (equation (2)), we set the length of a node along the y -axis to be equal to 1 unit length (its width being set to 0.2 units). For simplicity, we chose equal activation energies (equation (1)) and took $kT = 1$.

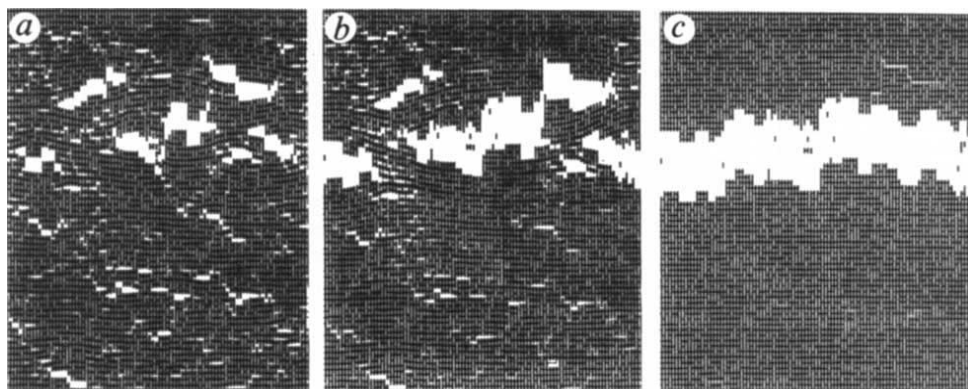


Fig. 2 Results of the simulations for $K_x = K_y = 20$ ($kT = 1$, U_i = constant for all the bonds). *a*, Long before failure; *b*, close to failure; *c*, at failure.

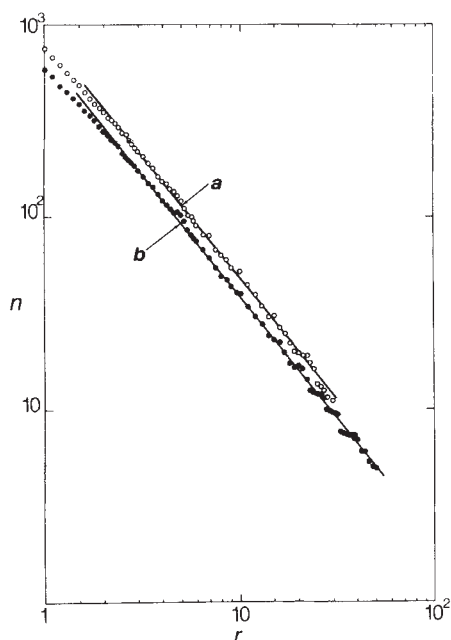


Fig. 3 log-log dependence of n on r (see equation (4)), where n is the number of yardsticks of size r needed to cover the perimeter of a crack; r is in units of the width of a node along the x -axis. Curve a : $K_x = K_y = 20$; curve b : $K_x = 200$, $K_y = 20$.

Figure 2 shows a two-dimensional network with $K_x = K_y = 20$, at different stages of our simulations. Long before failure (Fig. 2a), small cracks are observed which grow in number. As failure is approached (Fig. 2b), these cracks merge together, giving rise to a smaller number of larger cracks. Note that all the cracks at the top of Fig. 2a and b have the same 'dog' shape and already suggest self-similarity properties. At failure (Fig. 2c), only one crack is present, which extends throughout the network along the x -axis. All the other cracks have either been incorporated into the latter or have been closed through relaxation of the remaining elastic bonds towards their unstretched state. The fraction of broken bonds in Fig. 2c is $\sim 30\%$.

The degree of irregularity of a crack perimeter is given by its fractal dimension D (ref. 1), obtained by measuring the perimeter length using yardsticks of different sizes. The perimeter is a fractal if

$$n \sim r^{-D} \quad (4)$$

where n is the number of yardsticks of size r needed to cover the entire perimeter. (Because, in general, one cannot cover the entire perimeter with an integral number of yardsticks, the termination is handled by taking the straight line segment between the current point and the end point of the perimeter. The ratio of the segment length to the yardstick length is then added to the yardstick count¹².) Curve a of Fig. 3 shows the log-log dependence of n on r for the perimeter of the 'infinite' crack in Fig. 2c. Fitting a straight line in the range $2 < r < 30$ leads to a slope $D = 1.27 \pm 0.02$. A similar value of D , although for a smaller size range of r , has been obtained for the perimeters of the finite cracks in Fig. 2b.

An alternative statistical measure of D for an ensemble of finite cracks examined with the same yardstick size r is defined by¹

$$P(r) \sim A(r)^{D/2} \quad (5)$$

where $P(r)$ is the perimeter and $A(r)$ is the area (for a given finite crack, the area A includes all the nodes and groups of nodes completely surrounded by the crack in question¹³). Equation (5) has been applied to the cracks in Fig. 2a and b by determining the value of D which, for a given r , minimizes

the standard deviation for the ratios $P(r)/A(r)^{D/2}$. In the range $1 < r < 8$, we find $D = 1.31 \pm 0.15$. Although the statistical error is large because of the small number of cracks investigated, the latter value of D is consistent with that obtained using equation (4).

The range of D values achievable by the model can be easily determined. In the limit of large kT , p_i is constant (see equation (1)) and the crack corresponds to a two-dimensional percolation cluster whose exterior perimeter (hull) has a fractal dimension of ~ 1.75 . In the limit $kT = 0$, the crack develops horizontally from a small nucleus and $D = 1$. For finite kT , D also depends on K_x and K_y . Computer simulation results for $kT = 1$ with $K_x = K_y = 10$ and $K_x = 200$, $K_y = 20$ (see Fig. 3, curve b) lead to D values which are identical, within experimental error, to that (1.27 ± 0.02) found for the initial choice $K_x = K_y = 20$. This indicates that for intermediate values of kT , the fractal dimensionality D is insensitive to model details and may take on a universal value for all finite, non-zero values of kT .

Admittedly, our model may be only a crude representation of real experimental systems. For example, the model in its present form does not allow for out-of-plane buckling phenomena or in-plane distortion effects. Nevertheless, we believe that these complications should be minimal in the perfectly ordered and oriented polymeric systems for which the present model was originally devised⁸. Concerning the comparison of our D values with experimental data, we note that for crushed glass, $D = 2.35$ (ref. 14), whereas for fractured steel the fractal dimensional increment D' ($D' = D - 1$) was found to be 1.27 (ref. 15). Although results in two dimensions cannot be extrapolated to three dimensions, it is interesting that the latter experimental values are consistent with our prediction $D = 1.27$ for a surface, if we assume D (three dimensions) $= D$ (two dimensions) $+ 1$.

Received 30 September 1985; accepted 16 January 1986.

1. Mandelbrot, B. B. *The Fractal Geometry of Nature*, 1-468 (Freeman, San Francisco, 1982).
2. Niemeyer, L., Pietronero, L. & Wiesman, H. J. *Phys. Rev. Lett.* **52**, 1033-1036 (1984).
3. Stanley, H. E. *J. Phys. A* **10**, L211-L220 (1977).
4. Kapitulnik, A., Aharony, A., Deutscher, G. & Stauffer, D. *J. Phys. A* **16**, L269-L274 (1983).
5. Family, F. *Phys. Rev. Lett.* **51**, 2112-2115 (1983).
6. Termonia, Y. & Meakin, P. *Phys. Rev. Lett.* **54**, 1083-1086 (1985).
7. Avnir, D., Farin, D. & Pfeifer, P. *Nature* **308**, 261-263 (1984).
8. Termonia, Y., Meakin, P. & Smith, P. *Macromolecules* **18**, 2246-2252 (1985).
9. Kausch, H. H. *Polymer Fracture*, 1-332 (Springer, Berlin, 1978).
10. Dobrodumov, A. V. & El'yashevich, A. M. *Sov. Phys. Solid State* **15**, 1259-1260 (1973).
11. de G. Allen, D. N. *Relaxation Methods*, 1-230 (McGraw-Hill, New York, 1954).
12. Farin, D., Peleg, S., Yavin, D. & Avnir, D. preprint, Hebrew Univ. of Jerusalem (1985).
13. Voss, R. F. *J. Phys. A* **17**, L373-L377 (1984).
14. Avnir, D. & Farin, D. *J. chem. Phys.* **79**, 3566-3571 (1983).
15. Mandelbrot, B. B., Passoja, D. E. & Paullay, A. J. *Nature* **308**, 721-722 (1984).

Detection of hail by dual-polarization radar

A. J. Illingworth*, J. W. F. Goddard† & S. M. Cherry†

* Department of Physics, University of Manchester Institute of Science and Technology, PO Box 88, Manchester M60 1QD, UK

† Rutherford Appleton Laboratories, Chilton, Oxfordshire OX11 0QX, UK

Hail causes considerable damage to property and agriculture and a simple, unambiguous method of determining which convective storms contain hail would be of great use for short-term weather forecasting, and would also find application in the study of the mechanism of hailstone growth. Previous radar methods of hail detection¹⁻³ have not proved reliable, but here we report results of a new technique based on the measurement of differential reflectivity using dual polarization radar and suggest an additional

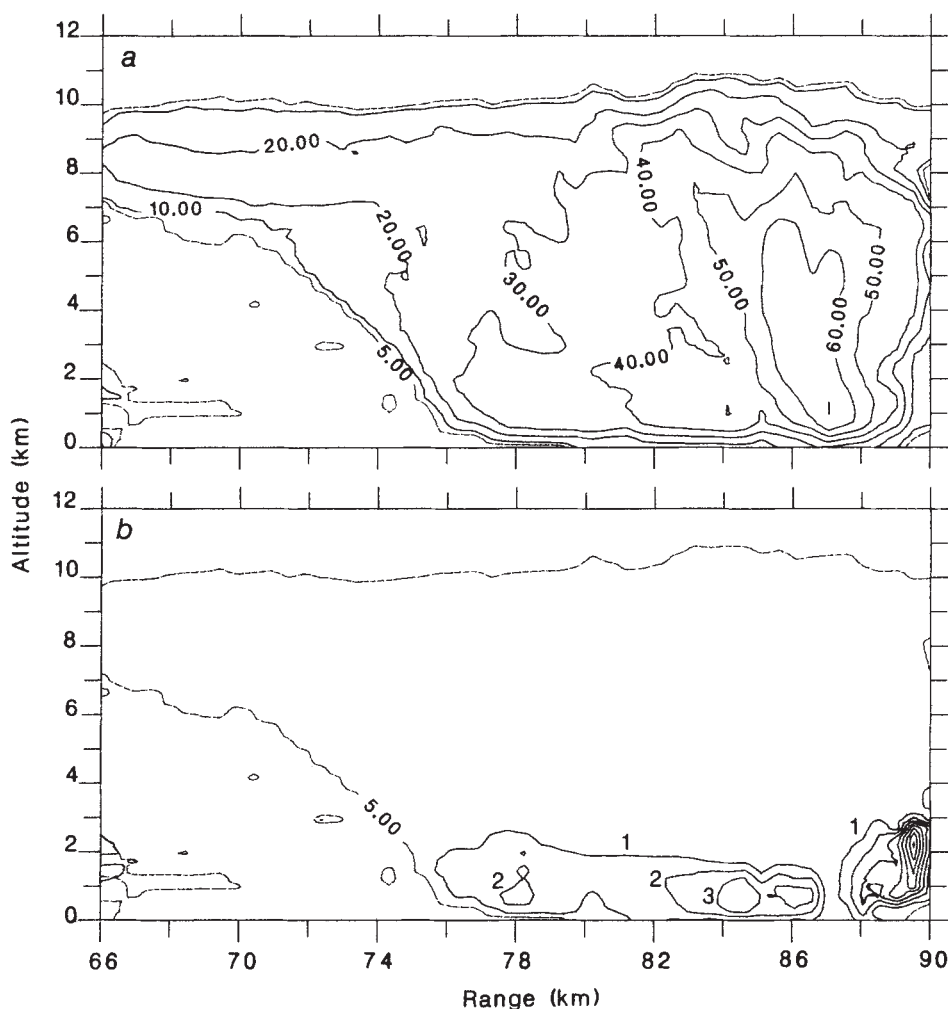


Fig. 1 Contour plots of reflectivity Z (a) and differential reflectivity Z_{DR} (b) obtained from a vertical scan through an intense convective storm at 13:36 UT, 6 July 1983. *a*, Contours of Z are drawn every 10 dBZ above $1 \text{ mm}^6 \text{ m}^{-3}$, with an additional dotted 5-dBZ contour. *b*, Contours of Z_{DR} are plotted at 1-dB intervals starting at 1 dB. The dotted 5-dBZ contour from *a* is included, to delineate the extent of the echo. The positive values of Z_{DR} below 2 km altitude are due to raindrops falling with their horizontal axis larger than the vertical. In contrast to this is a narrow region at 87 km (<1 km wide) at 87 km range where near-zero values of Z_{DR} extend down to the ground. We believe that this region contains tumbling hail falling to the ground.

criterion for hail recognition. Hail is known to occur in vigorous convective storms in which the effective radar reflectivity, Z , measured by conventional radar is high. If the hail reaches the ground it is usually restricted to long narrow swathes⁴, often about 1 km wide but up to 50 km long, accompanied by surrounding precipitation in the form of heavy rain. The differential reflectivity technique involves comparing the radar reflectivity factor for horizontally (Z_H) and vertically (Z_V) polarized radiation; the comparison provides a measure of the mean shape of the precipitation particles. Close to the ground the values of Z are high for both hail and heavy rain, but because the large raindrops are oblate and fall with their horizontal diameter greater than the vertical, the value of Z_H in the rain exceeds that of Z_V . The smaller regions where hail is present can be clearly distinguished from the neighbouring heavy rain because, although individual hailstones may not be spherical, they tumble as they fall and so result in equal values of Z_H and Z_V .

For meteorological radars the power scattered back by common precipitation particles is proportional to the sixth power of the particle size and, although the particles may be frozen or liquid and may be non-spherical, it is usual to express the return as the equivalent radar reflectivity factor, Z , defined as the summation per unit volume of the diameter, raised to the sixth power, of spherical water drops which would scatter the same power as that actually measured. Z is usually quoted in decibels above $1 \text{ mm}^6 \text{ m}^{-3}$. A full review of meteorological applications of radar is provided by Browning⁵.

The new method of hail detection relies on a measurement

of the differential reflectivity, Z_{DR} , defined in decibels as

$$Z_{DR} = 10 \log_{10} (Z_H / Z_V)$$

The radar used in this study is fully described by Cherry and Goddard⁶. Situated at Chilbolton in southern England, it has an antenna of 25 m diameter, giving a 0.25° beamwidth at the 10 cm operating wavelength. Pulses with horizontal and vertical polarizations are transmitted (and received) alternately with a pulse repetition frequency (PRF) of 610 Hz. The precipitation forms an incoherent target with the values of Z fluctuating as the hydrometeors reshuffle in space, but this choice of PRF ensures adequate correlation of Z between sequential samples so that Z_{DR} may be estimated accurately. Each range-gated data sample comprises a spatial average over 300 m (four pulse volumes), which is time averaged over 64 transmitted pulse pairs. The worst case errors in measuring Z and Z_{DR} are estimated to be 0.7 dBZ and 0.2 dB respectively.

A vertical section through a vigorous convective storm is shown in Fig. 1. In Fig. 1a, contours of the conventional reflectivity Z are drawn every 10 dBZ, from 10 dBZ to 70 dBZ above $1 \text{ mm}^6 \text{ m}^{-3}$. In Fig. 1b, contours of Z_{DR} , observed simultaneously, are drawn at 1-dB intervals. The dotted Z contour at 5 dBZ in *a* and *b* delineates the overall extent of the echo. On this day the 0°C isotherm was at about 3 km altitude, and (as is typical for convective clouds⁷) values of Z_{DR} at higher altitudes in Fig. 1b are very close to zero, indicating randomly tumbling ice particles. Closer to the ground where it is warmer than 0°C , rain is to be expected, and as the rain intensity

increases the drops become larger and more oblate, leading to larger values of Z_{DR} ; assuming a Marshall–Palmer raindrop size distribution⁸, calculations show⁹ that Z_{DR} should exceed 1 dB when the Z value exceeds 35 dBZ. In contrast to this is a region at a range of about 87 km where low values of Z_{DR} extend down to the ground. For a width of about 600 m (that is, over two neighbouring samples), a Z_{DR} below 0.5 dB accompanied by a Z of 65 dBZ extends down to a radar elevation of zero degrees. For monodispersed raindrops this value of Z_{DR} implies diameters less than 2 mm, which would need to be present in the unrealistic concentration of $500,000\text{ m}^{-3}$ to give the observed Z . This corresponds to rainfall rates of thousands of millimetres per hour, and any other distribution would require even higher concentrations. The only plausible explanation is that the precipitation is hail which is nearly spherical or tumbles as it falls. The hail must be large, and with a high terminal velocity, so that although the surface is wet it has not melted sufficiently to form an oblate-shaped water coating. A similar but less extreme case can be seen in Hall *et al.*⁷.

To monitor hail over a large area, the radar could be scanned in azimuth at low elevation so that in the summer only precipitation at temperatures much above 0°C is sampled. If regions where Z is above 45 dBZ are considered, then any region where Z_{DR} is less than 0.7 dB can be identified as hail reaching the ground. Hail-swaths could be tracked by monitoring the movement, in successive scans, of such low- Z_{DR} cores embedded in regions of both high Z and Z_{DR} .

The technique outlined above would not result in false alarms. However, the precise shape and fall-mode of hail is not well known and if aspherical hail falls with a degree of common alignment, then Z_{DR} might have a local minimum but not fall below the 0.7 dB level. In 1986 the Chilbolton radar will be able to detect the orthogonal return in addition to the co-planar one. This information may provide additional corroborating evidence for hail. The linear depolarization ratio, L_{DR} , for vertically polarized transmitted radiation is defined as

$$L_{DR} = 10 \log_{10} (Z_{V,H}/Z_V)$$

where $Z_{V,H}$ is the horizontally polarized return. L_{DR} values of less than -20 dB have been measured¹⁰ in rain at a 3.2-cm wavelength. The orthogonal return is very low in amplitude because it is believed¹¹ that raindrops fall with their axes aligned in the vertical, with canting angles of less than 1° . Hail is generally aspherical and computations¹² show that randomly tumbling wet oblate spheroids with an axial ratio of 0.7 should have an L_{DR} greater than -20 dB. If this is so, the L_{DR} hail signature is simple: summertime values of L_{DR} above -20 dB close to the ground must be due to hail. The extreme case of spherical hail would escape L_{DR} -detection, but would give a clear zero Z_{DR} signal. At present there are no reports of measurements of L_{DR} at a 10-cm wavelength, but analysis of data at 3 cm (supplied by P. H. Herzegh, personal communication) confirms that, although propagation problems affect the absolute values of L_{DR} , a local increase of about 6 dB appears to coincide with a hail shaft.

The presence of hail seems to be the only physically realistic explanation of the localized region of low Z_{DR} embedded in a more extensive region where both Z and Z_{DR} are high. Although it has not been possible to obtain independent ground-based observations in the 500-m-wide region shown in Fig. 1, a long series of comparisons¹³ of the radar data with simultaneous raindrop size distributions measured at the ground reveals that such high Z -low Z_{DR} combinations are not found in rainfall. More extensive observations with the Chilbolton radar scanning in azimuth at low elevation should enable the tracking of low- Z_{DR} cores and any accompanying anomalous- L_{DR} regions, and thus increase the probability of obtaining independent ground-based confirmation of the hail identification.

We acknowledge support from the Meteorological Office and EOARD through grant AFOSR-85-0188. We thank R. E.

Carbone, P. H. Herzegh, V. N. Bringi and T. A. Seliga for supplying us with L_{DR} data from the Maypole '84 program.

Received 11 November 1985; accepted 12 February 1986.

- Geotis, S. G. *J. appl. Met.* **2**, 270–275 (1963).
- Carbone, R. E. *et al. Bull. Am. met. Soc.* **54**, 921–927 (1973).
- Barge, B. L. *McGill Univ. Sci. Rep.* MW-71 (Montreal 1972).
- Morgan, G. M. & Towery, N. G. *J. appl. Met.* **14**, 763–770 (1975).
- Browning, K. A. *Rep. Prog. Phys.* **41**, 761–806 (1978).
- Cherry, S. M. & Goddard, J. W. F. *URSI Conf.*, Bournemouth 49–54 (RAL, 1982).
- Hall, M. P. M., Goddard, J. W. F. & Cherry, S. M. *Radio Sci.* **19**, 132–140 (1984).
- Marshall, J. S. & Palmer, W. J. *Met. S.* **5**, 165–166 (1948).
- Illingworth, A. J., Goddard, J. W. F. & Cherry, S. M. *Am. met. Soc. 22nd Radar Met. Conf.*, Boston, 145–150 (American Meteorological Society, 1984).
- Browne, I. C. & Robinson, N. P. *Nature* **170**, 1078–1079 (1952).
- Beard, K. V. & Jameson, A. R. *J. atmos. Sci.* **40**, 448–454 (1983).
- Atlas, D., Kerker, M. & Hitschfeld, W. *J. atmos. terr. Phys.* **3**, 108–119 (1953).
- Goddard, J. W. F. & Cherry, S. M. *Am. met. Soc. 22nd Radar Met. Conf.*, Boston 352–357 (American Meteorological Society, 1984).

A new geochronometer using lanthanum-138

S. Nakai, H. Shimizu & A. Masuda*

Laboratory for Rare Earth Element Microanalysis, Department of Chemistry, University of Tokyo, Hongo, Tokyo, 113 Japan

We have applied a new geochronometer, based on the decay of ^{138}La to ^{138}Ba , to a sample of Amitsoq gneiss from West Greenland. Using a value for the electron-capture decay constant, λ_{EC} , of $4.44 \times 10^{-12}\text{ yr}^{-1}$, the resulting age ($2,408 \pm 24\text{ Myr}$) is in good agreement with that from Sm–Nd systematics ($2,410 \pm 54\text{ Myr}$). Application of this geological clock is limited to REE (rare earth element)-rich minerals, but its merit is that, for Archaean rocks, the age can be evaluated from one specimen on the assumption that the initial Ba isotope ratios are effectively constant.

^{138}La decays to ^{138}Ce and ^{138}Ba by β -decay and electron capture, respectively. Tanaka and Masuda¹ developed a dating method which employed the ^{138}La – ^{138}Ce decay. Preliminary trial by Masuda and Nakai² demonstrated the possible geochronological utility of the ^{138}La – ^{138}Ba decay. Our recent access to a better mass spectrometer, and improvement in chemical procedures, have enabled us to advance towards realization of a La–Ba clock.

For REE-rich minerals such as allanite and monazite, in which the La/Ba abundance ratios are higher than 3×10^2 , the accumulation of radiogenic ^{138}Ba from Precambrian times is sufficiently large to let us measure their formation ages using the following equation:

$$\begin{aligned} (^{138}\text{Ba}/^{137}\text{Ba})_p &= (^{138}\text{Ba}/^{137}\text{Ba})_0 \\ &+ \lambda_{EC}/\lambda_{\text{total}} \times (^{138}\text{La}/^{137}\text{Ba})_p \\ &\times [\exp(\lambda_{\text{total}} T) - 1] \end{aligned}$$

where subscript 'p' refers to present, subscript '0' to the time of formation, λ_{EC} is the electron-capture partial decay constant of ^{138}La and λ_{total} the total decay constant of the same nuclide. In general, $(^{138}\text{Ba}/^{137}\text{Ba})_0$ can be regarded as essentially constant, because the contribution of radiogenic ^{138}Ba to non-radiogenic ^{138}Ba in ordinary minerals, even after $4.5 \times 10^9\text{ yr}$, is smaller than the range of experimental errors for measurement of $^{138}\text{Ba}/^{137}\text{Ba}$ ratios and is thus negligible at the present stage of our research. (The cosmic abundance ratio for La/Ba is ~ 0.1 and the isotopic percentage of ^{138}La with half-life $\sim 10^{11}\text{ yr}$ is 0.089, while that of ^{138}Ba is 71.70. If the precision of La/Ba dating is enhanced

* To whom correspondence should be addressed.

by future progress in mass spectrometry, it may become necessary to admit the dependence of $(^{138}\text{Ba}/^{137}\text{Ba})_0$ on sample age and composition.) The published values for the half-life of ^{138}La scatter widely; we have therefore compared our La-Ba ages with Sm-Nd mineral isochron ages for the same samples.

The barium blank from the chemical procedures can be considered as having the same $^{138}\text{Ba}/^{137}\text{Ba}$ ratio as the initial ratio for the reason mentioned above, which constitutes a unique merit of the dating method under consideration. As noted previously², provided that the experimental procedure is designed to have the same Ba blank effects for both the Ba isotopic composition measurement and the Ba abundance determination, the effect of blank contamination is to move points along the isochron line in the $^{138}\text{Ba}/^{137}\text{Ba}$ versus $^{138}\text{La}/^{137}\text{Ba}$ diagram. It follows that no Ba blank corrections are needed for the Ba isotopic ratios and Ba abundances thus obtained. The new geochronometer has been applied to two sets of samples of Precambrian age. The first set of minerals is from the Amîtsoq gneiss (GGU110999) of West Greenland, from which allanite, epidote and sphene were used for dating. The second set comprises allanite, apatite and sphene from the Mustikkamäki pegmatite of Finland.

Barium and REE were determined by stable isotope dilution. The Ba blank from the separation procedure was ~2 ng. No blank correction was made for the reason previously mentioned. The overall blank for Nd was ~1 ng, which is negligible.

Ba and Nd isotopic compositions were measured with a VG 354 mass spectrometer equipped with five Faraday collectors; abundances of Ba, La, Sm and Nd were determined with a JEOL JMS-05RB mass spectrometer. Barium isotopes were measured using Ta-Re-Ta triple filaments. To correct the variation due to mass discrimination, we normalized all the measured isotopic ratios to $^{136}\text{Ba}/^{137}\text{Ba} = 0.6996$. The measurements of $^{138}\text{Ba}/^{137}\text{Ba}$ gave 6.38969 ± 12 (2 σ) for a Merck BaCl_2 reagent (Art 1716), 6.38997 ± 22 (2 σ) for JB-1 standard basalt and 6.38972 ± 27 (2 σ) for BCR-1 standard basalt. The results show the uniformity of Ba isotopic ratios of ordinary rocks within experimental uncertainties, and endorse our suggestion that the initial $^{138}\text{Ba}/^{137}\text{Ba}$ ratio can be taken as invariable. Our results are nearly in agreement with those of Eugster *et al.*³ and Lewis *et al.*⁴. (The ratio for reagent Ba, 6.38969 ± 12 , can be taken as representing a 'fixed spot' value.) Neodymium was measured as Nd^+ on Re triple filaments. All the measured isotopic ratios were normalized to $^{146}\text{Nd}/^{144}\text{Nd} = 0.7219$. Our $^{143}\text{Nd}/^{144}\text{Nd}$ ratio for La Jolla Nd standard reagent was 0.511849 ± 6 (2 σ).

Results are shown in Table 1 and Figs 1-3. The isochron lines were fitted using the York method⁵. Uncertainties (2 σ) for $^{138}\text{La}/^{137}\text{Ba}$ and $^{147}\text{Sm}/^{144}\text{Nd}$ were estimated at 0.5%. We used $1.56 \pm 0.05 \times 10^{11}$ yr for the electron-capture partial half-life ($\lambda_{\text{EC}} = 4.44 \pm 0.15 \times 10^{-12} \text{ yr}^{-1}$), and $1.03 \pm 0.02 \times 10^{11}$ yr for the total half-life of ^{138}La ($\lambda_{\text{total}} = 6.73 \pm 0.13 \times 10^{-12} \text{ yr}^{-1}$), as reported by Sato and Hirose⁶. Figure 1 shows a La-Ba isochron plot for the allanite, epidote and whole-rock sample from Amîtsoq gneiss specimen GGU110999 (analyses in Table 1). The three data points define an age of $2,408 \pm 24$ (2 σ) Myr. This

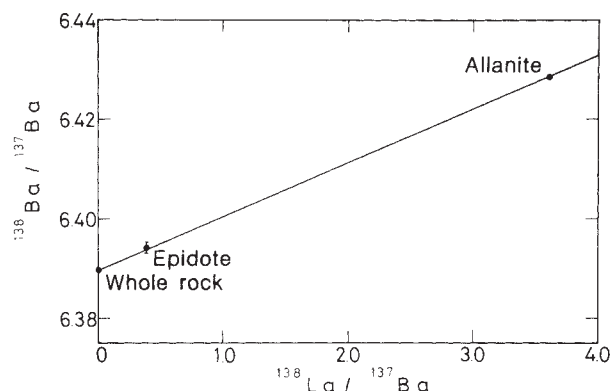


Fig. 1 La-Ba mineral isochron plot for GGU110999 from Amîtsoq gneiss, West Greenland. The isochron yields an age of $2,408 \pm 24$ (2 σ) Myr with an initial $^{138}\text{Ba}/^{137}\text{Ba}$ ratio of 6.38968 ± 28 (2 σ) ($\lambda_{\text{EC}} = 4.44 \times 10^{-12} \text{ yr}^{-1}$).

result agrees well with the Sm-Nd mineral isochron age of $2,410 \pm 54$ (2 σ) Myr (Fig. 2). This agreement is considered not only to prove the validity of La-Ba dating, but also to support the decay constants, and in particular the λ_{EC} value, presented by Sato and Hirose⁶. (The La-Ba age is far more sensitive to λ_{EC} than to λ_{total} or λ_{β} .)

Baadsgaard *et al.*⁷ have carried out thorough investigations of mineral isotopic age relationships in Amîtsoq gneisses, using U-Th-Pb, Pb-Pb, Rb-Sr and K-Ar systematics. A complicated history of variable mineral response to polymetamorphism is recorded in six rocks, including GGU110999. Based on all the radiometric evidence, it has been concluded that three major metamorphic events affected the Amîtsoq gneisses at ~3,600, 2,500 and 1,550 Myr. (According to our Sm-Nd data on GGU110999 whole rock, its model Nd age is estimated to be 3,810 Myr.) Our results obtained from La-Ba and Sm-Nd systematics are in keeping with the conclusion of Baadsgaard *et al.*⁷ from Rb-Sr mineral analyses, that allanite and sphene appear to have responded to metamorphism most recently at ~2,200-2,600 Myr. The K-Ar ages for biotite and hornblende⁷ in GGU110999 are also concordant, at 2,317 and 2,342 Myr, respectively. The concordance of mineral ages is not perfect: the Rb-Sr system in epidote was affected⁷ by an event at ~1,550 Myr, whereas the GGU110999 epidote falls on the Sm-Nd isochron at 2,410 Myr (Fig. 2), together with allanite and sphene.

We have also investigated the Mustikkamäki pegmatite of Finland. The sphene and allanite from this pegmatite define an isochron age of $1,694 \pm 42$ (2 σ) Myr. This La-Ba age is younger than the Sm-Nd age, $1,820 \pm 29$ (2 σ) Myr (Fig. 3). Apparently the geochronology of the Mustikkamäki pegmatite is more complicated than that of the Amîtsoq gneiss, but interpretation based on the geological background is difficult at present (O. Kouvo, personal communication). (For the La-Ba age to be strictly consistent with the Sm-Nd age for this pegmatite, λ_{EC} must equal $4.13 \times 10^{-12} \text{ yr}^{-1}$, which yields 2,588 in place of

Table 1 La-Ba and Sm-Nd isotopic data

Sample	La (p.p.m.)	Ba (p.p.m.)	$^{138}\text{La}/^{137}\text{Ba}^*$	$^{138}\text{Ba}/^{137}\text{Ba}^\dagger$	Sm (p.p.m.)	Nd (p.p.m.)	$^{147}\text{Sm}/^{144}\text{Nd}^*$	$^{143}\text{Nd}/^{144}\text{Nd}^\dagger$
Amîtsoq gneiss (GGU110999)								
Whole rock	11.57	163.1	0.00056	6.38963 ± 36	2.500	13.07	0.1156	0.510593 ± 8
Allanite	1.680×10^4	36.65	3.607	6.42856 ± 30	1,236	1.252×10^4	0.05964	0.509725 ± 11
Epidote	599.0	11.98	0.3919	6.39422 ± 80	68.85	520.2	0.07997	0.510045 ± 8
Sphene					384.9	1,239	0.1878	0.511773 ± 8
Mustikkamäki pegmatite								
Allanite	3.218×10^4	62.34	4.058	6.42033 ± 30	2,010	1.622×10^4	0.07489	0.511101 ± 8
Apatite					50.11	187.2	0.1618	0.512157 ± 8
Sphene	187.1	110.2	0.00973	6.38972 ± 30	837.7	1,061	0.4779	0.515910 ± 8

* Uncertainties are <0.5%. † Errors are 2 σ of the mean.

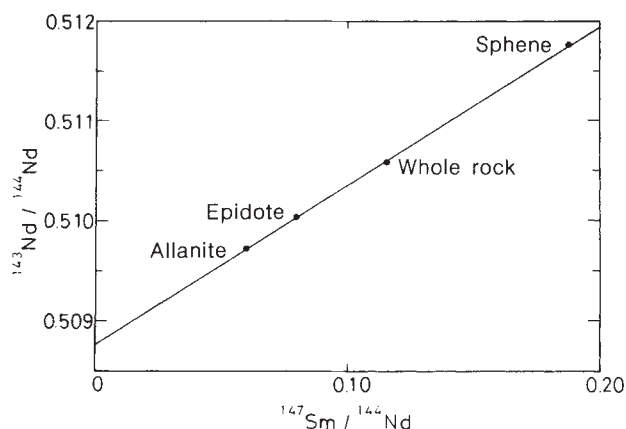


Fig. 2 Sm-Nd mineral isochron plot for 110999 obtained from aliquots of the same sample solution as that used for La-Ba analysis. The isochron yields an age of $2,410 \pm 54$ (2 σ) Myr with an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.508773 ± 34 (2 σ) ($\lambda_a = 6.54 \times 10^{-12} \text{ yr}^{-1}$).

2,408 Myr for the GGU110999 La-Ba age.) The above discrepancy between 1,694 and 1,820 Myr is considered to be related to the thermal or metamorphic history of the district concerned and to geochemical mobility of the relevant elements. Some isotopic systems may remain undisturbed through a metamorphic event, while others are disturbed or even completely reset. The response of different isotopic systems is a function of the severity of the metamorphism, and of the geochemical behaviour of the parent and daughter elements in relation to the particular mineral assemblages and reactions associated with the metamorphism.

Apart from the electron capture of ^{138}La , there are two nuclear reactions which produce ^{138}Ba in terrestrial minerals. One is the spontaneous fission of ^{238}U with partial decay constant $\lambda_f = 8.46 \times 10^{-17} \text{ yr}^{-1}$ (ref. 8), and fission yields for ^{138}Ba and ^{137}Ba of 7.2% and 6.6% (ref. 9). Minerals in which the abundance of uranium is >400 times that of barium suffer from the effect of fission and the La-Ba age will change slightly. It is evident that the U contents in our samples are low enough to neglect the fission effect. Another reaction to be considered is the α -decay of ^{142}Ce , the effect of which is marginal for La-Ba dating, although the half-life of ^{142}Ce is not precisely known¹⁰⁻¹². If the shortest reported half-life ($5 \times 10^{15} \text{ yr}$; ref. 10) is adopted, the La-Ba ages reported above are $\sim 0.8\%$ too high. But if the longer half-life ($>5 \times 10^{16} \text{ yr}$; ref. 12) is employed, the α -decay effect is nearly negligible. (A half-life of $9 \times 10^{18} \text{ yr}$ has also been reported.)

La-Ba dating is applicable to monazite, allanite, Ba-poor epidote, and other REE-rich minerals. The common occurrence

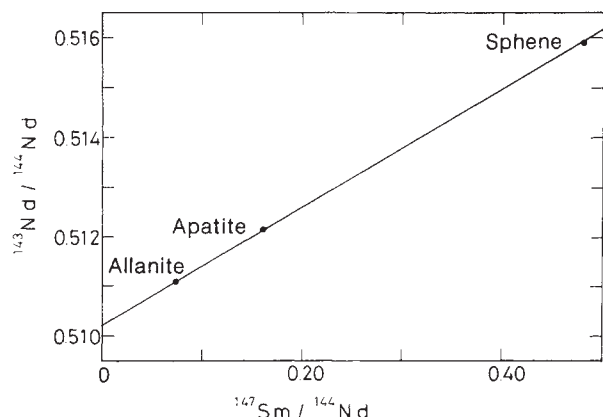


Fig. 3 Sm-Nd mineral isochron plot for the Mustikkamäki pegmatite, Finland. The isochron yields an age of $1,820 \pm 29$ (2 σ) Myr with an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.510207 ± 26 (2 σ).

of epidote as a product of low-grade metamorphic events suggests that La-Ba dating may be used to date such events in ancient shield areas. The La/Ba ratios in sphene and apatite appear sometimes to be high enough to allow La-Ba dating. The age of allanite can be determined with one (mm-sized) grain. Although this geochronometer works only for a limited number of minerals, it will have much significance for studies of the evolution of the continental crust, especially in the Precambrian.

We thank Professor H. Baadsgaard (University of Alberta) and Dr O. Kouvo (Geological Survey of Finland) for donation of samples, Professor D. Bridgwater (Copenhagen University) for helpful information, and S. Tasaki for help in chemical separation. Permission to publish results on material from the Geological Survey of Greenland (GGU110999) is acknowledged. This research was supported by grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

Received 16 September 1985; accepted 30 January 1986.

1. Tanaka, T. & Masuda, A. *Nature* **300**, 515-518 (1982).
2. Masuda, A. & Nakai, S. *Geochim. J.* **17**, 313-314 (1983).
3. Eugster, O., Tera, F. & Wasserburg, G. J. *J. geophys. Res.* **74**, 3897-3908 (1969).
4. Lewis, R. S., Anders, E., Shimamura, T. & Lugmair, G. W. *Science* **222**, 1013-1015 (1983).
5. York, D. *Can. J. Phys.* **44**, 1079-1086 (1966).
6. Sato, J. & Hirose, T. *Radiochem. radioanalyt. Lett.* **46**, 145-152 (1981).
7. Baadsgaard, H., Lambert, R. St J. & Krupicka, J. *Geochim. cosmochim. Acta* **40**, 513-527 (1976).
8. Galliker, D., Hugentobler, E. & Hahn, B. *Helv. phys. Acta* **43**, 593-606 (1970).
9. Ishimori, T. *Nippon Genshiryoku Kenkyusho Kenkyu Hokoku* **1102**, 95-97 (1966).
10. Reizler, W. & Kauw, G. Z. *Naturf.* **12A**, 665-666 (1957).
11. Seftle, F. E., Stern, T. W. & Alekna, V. P. *Nature* **184**, 630 (1959).
12. MacFarlane, R. D. & Kohman, T. P. *Phys. Rev.* **121**, 1758-1769 (1961).

Cosmogenic helium in a terrestrial igneous rock

Mark D. Kurz

Chemistry Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

High $^3\text{He}/^4\text{He}$ ratios in terrestrial samples are generally interpreted as indicating the presence of primitive or primordial gas^{1,2}. Despite the ubiquitous presence of cosmogenic noble gases in meteorites, cosmogenic helium isotopic signatures have never been observed in terrestrial samples³ and have, for the most part, been considered to be insignificant on the Earth. I present here new helium isotopic measurements on samples from the Kula formation of Haleakala volcano (Hawaii) that are best explained by an *in situ* cosmogenic origin for a significant fraction of the ^3He . Results from crushing and stepwise heating experiments, and consideration of the exposure age of the sample at the surface and the cosmic-ray fluxes strongly support this hypothesis. Although crustal cosmogenic helium has been proposed previously^{4,5}, this represents its first unambiguous identification in a terrestrial sample.

Haleakala volcano, which constitutes the eastern end of the island of Maui, is a shield volcano consisting of tholeiitic lavas unconformably overlain by later alkali basalts^{6,7}. The samples discussed here are alkali basalts primarily from the Kula formation, and were erupted between 500,000 and 800,000 yr ago^{8,9}. Two of them (H85-10 and H65-4) are drill-core samples^{10,11} that are overlain by 160 m of younger Hana formation basalts. The third sample (HS85-3) was collected from the upper 50 cm of a 1.5-m thick surface lava flow at White Hill, near the summit of Haleakala (at elevation 3,200 m); it is a section of the Kula formation that was not buried by the later Hana series⁶. Kaneoka and Takaoka¹² reported high $^3\text{He}/^4\text{He}$ ratios, up to 37 times the atmospheric value (R_a) from the same area, a result that was later confirmed by Rison and Craig¹³. As shown below, these

Table 1 Helium analyses of basalts from Haleakala Volcano

Sample	Description	Depth	Phase analysed	Crushed		Powder heated		1–2 mm grains heated	
				^4He ($\text{cm}^3 \text{ STP g}^{-1}$)	$^3\text{He}/^4\text{He}$ (R/R_a)	^4He ($\text{cm}^3 \text{ STP g}^{-1}$)	$^3\text{He}/^4\text{He}$ (R/R_a)	^4He ($\text{cm}^3 \text{ STP g}^{-1}$)	$^3\text{He}/^4\text{He}$ (R/R_a)
HS85-3	Ankaramite, White Hill	Surface	Olivine	5.76×10^{-9}	8.2 ± 0.2	$1.82 \times 10^{-9*}$	417*	$[7.88 \times 10^{-9}]$	[106.4]
			Olivine	1.22×10^{-8}	8.2 ± 0.1				
	Ankaramite with 20% vesicles and 25% phenocrysts. The phenocryst population consists of 60% subhedral clinopyroxene (2–7 mm), and 40% subhedral to euhedral olivine. Zoning and partial resorption of clinopyroxenes is evident in thin section. Phenocrysts contain abundant spinel inclusions and some melt inclusions		Olivine					1.52×10^{-8}	62 ± 2
			Olivine					$1.44 \times 10^{-8*}$	68.4*
			Clinopyroxene	3.15×10^{-9}	8.2 ± 0.1	$4.14 \times 10^{-9*}$	215.2*	$[3.56 \times 10^{-8}]$	[32.2]
			Clinopyroxene					$3.07 \times 10^{-8*}$	35.6*
			Matrix (holocrystalline basalt)					$1.66 \times 10^{-8*}$	26.7*
H65-4	Drill-core sample	164 m	Olivine	8.53×10^{-9}	8.3 ± 0.2			1.54×10^{-8}	8.00 ± 0.1
	Alkali basalt containing 1% vesicles and 5–6% phenocrysts; the phenocrysts consist of ~70% (2–3 mm) clinopyroxene, 30% (1–2 mm) olivine, and sparse plagioclase (see also refs 10,11).								
H85-10	Drill-core sample	249 m	Olivine	8.92×10^{-9}	8.1 ± 0.3	$5.34 \times 10^{-9*}$	4.89*		
	Alkali basalt containing ~3% 1–3-mm subhedral to euhedral olivine, 2% plagioclase and 1% clinopyroxene (see also refs 10,11)								
H1790-3	Surface		Olivine	2.38×10^{-8}	7.9 ± 0.1			3.56×10^{-8}	8.02 ± 0.2
	Ankaramite from 1790 flow of Haleakala								

Mineral separates were prepared by lightly crushing the sample in a stainless-steel mortar, handpicking the 1–2-mm size fraction, and sonic cleaning in dilute nitric acid, distilled water and acetone. The basalt matrix (sample HS85-3) was handpicked to eliminate any traces of alteration or phenocrysts, but was not treated with solvents (due to the porous nature of the matrix). In several instances, the powder remaining after crushing was recovered from the vacuum vessel, placed in an aluminium foil boat, and subjected to stepwise heating. The procedural blanks for crushing and heating during this study were $5.2 \pm 0.2 \times 10^{-11}$ (σ , sample standard deviation) $\text{cm}^3 \text{ STP } ^4\text{He}$ with $^3\text{He}/^4\text{He}$ ratios indistinguishable ($\pm 20\%$) from atmospheric. All samples were devoid of any alteration phases in thin section, hand specimen or in mineral separates.

* Sum of step-heating experiments (see Table 2). [], Sum of 'crushed' and 'powder heated' values from columns 1 and 2.

high $^3\text{He}/^4\text{He}$ ratios are due to cosmogenic ^3He within the samples rather than mantle-derived helium as suggested by these authors.

The $^3\text{He}/^4\text{He}$ ratios obtained by heating sample HS85-3 (Tables 1, 2) are higher than any previously reported value for terrestrial materials, and considerably higher than primordial meteoritic or even solar-wind helium. Experimental artefacts can immediately be eliminated as an explanation for these high $^3\text{He}/^4\text{He}$ ratios. Procedural blanks measured before and after each sample never deviated significantly from the mean blank value. No memory effects could produce the observed results because only terrestrial samples have been analysed in this laboratory, and no anomalous helium has ever been introduced into the furnace, crusher, vacuum lines or mass spectrometer. In addition, samples analysed both before and after HS85-3 (three of which are reported in Table 1) did not display these extreme ratios. Rayleigh fractionation of ^3He and ^4He is also quite unlikely given the large variability observed (between 8 and $2,600 \times R_a$). Finally, eight different analyses of this sample, using three different extraction methods (crushing, heating of powder, and heating of 1–2 mm grains; Table 1), yielded internally consistent results, which would not be produced by any experimental artefacts.

Contamination of the sample by anthropogenic tritium can also be ruled out given the large quantities of tritium that would be required. The amount of excess ^3He in all the HS85-3 mineral separates is $\sim 1 \times 10^{-12} \text{ cm}^3 \text{ STP } ^3\text{He g}^{-1}$. To produce this amount of ^3He would require, for example, that the rock consist of 40% water that had a tritium concentration of 1,000 TU (tritium units), which is clearly unrealistic. This possibility is conclusively ruled out by analysis of a surface sample from the 1790 flow on Haleakala (sample H1790-3; Table 1) which does not display anomalous ^3He , but has been exposed to all anthropogenic tritium.

Note also that the total $^3\text{He}/^4\text{He}$ ratio data are consistent, in a general way, with results of two other laboratories from the same location^{12,13}. Although the ratios reported here are significantly higher, any cosmogenic influence is strongly depth-dependent, being greater nearest the surface, so this difference could easily be explained by sampling geometry.

Several important aspects of the data in Tables 1 and 2 demonstrate why extreme ratios have not been observed previously. First, the extraction methods involved both crushing and heating in vacuum (discussed in detail elsewhere^{14,15}), which yield quite different results. In all cases presented here, helium released by crushing phenocrysts was within one standard deviation of 8.2 times atmospheric in $^3\text{He}/^4\text{He}$. However, for sample HS85-3, stepwise heating of the powder remaining after crushing released helium with extremely high $^3\text{He}/^4\text{He}$ ratios, up to 2,600 times atmospheric. This extreme internal isotopic variability is clearly illustrated by the step-heating pattern in Fig. 1. The highest $^3\text{He}/^4\text{He}$ ratios are released by heating the powder at low to intermediate temperatures. However, at higher temperatures progressively more ^4He is released and the $^3\text{He}/^4\text{He}$ decreases, approaching but not quite reaching the $^3\text{He}/^4\text{He}$ ratio released by crushing.

The stepwise heating pattern for another sample of Kula age is shown in Fig. 2. This sample differs from HS85-3 primarily in that it is overlain by 160 m of younger Hana basalts and is hence shielded from cosmic rays; the helium released by step-heating never exceeds the $^3\text{He}/^4\text{He}$ ratio of the crushing extraction. The release pattern is similar to Fig. 1 in that most of the ^4He is released at 1,350 °C, but in this case, the crushed helium and high-temperature-released helium are isotopically identical at 8 R_a .

The stepwise heating patterns of both olivine powder samples show that the lowest temperature step releases helium having a lower $^3\text{He}/^4\text{He}$ ratio than the successive step. The simplest

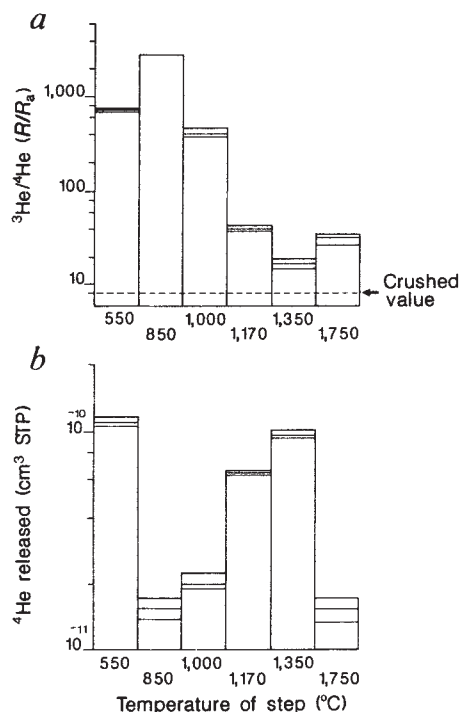


Fig. 1 Stepwise heating of Haleakala surface sample HS85-3, olivine powder. Each step represents the $^3\text{He}/^4\text{He}$ (a) and ^4He (b) released at the indicated temperature. The dashed line gives the $^3\text{He}/^4\text{He}$ ratio yielded by crushing *in vacuo*, which is attributed to inherited magmatic helium. The extremely high $^3\text{He}/^4\text{He}$ ratios (up to 2,600 R_a) released at lower temperature are the highest reported terrestrial values, and are attributed to cosmogenic helium. Error bars, 1σ uncertainty.

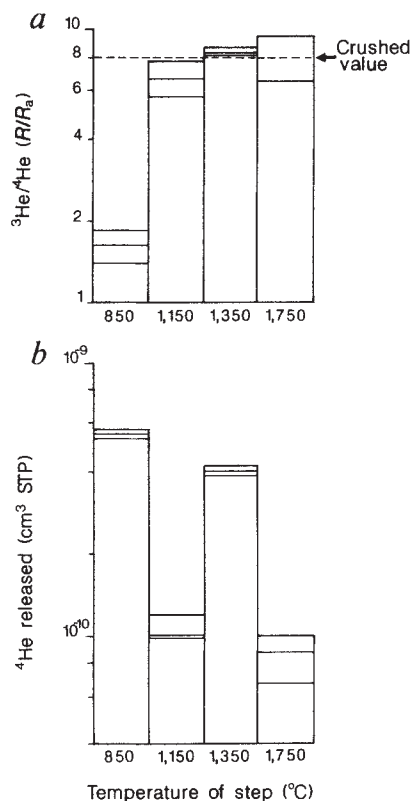


Fig. 2 Stepwise heating of olivine powder from drill-core sample H85-10. The lower $^3\text{He}/^4\text{He}$ ratio in the low-temperature step is attributed to radiogenic ^4He . In contrast to the release pattern of Fig. 1, the higher temperature steps are in equilibrium with the $^3\text{He}/^4\text{He}$ released by crushing (8 R_a), which is attributed to the inherited magmatic helium.

explanation for the low-temperature, low- $^3\text{He}/^4\text{He}$ component is radiogenic helium from the surface of the phenocrysts that are in contact with the basalt matrix, by implantation of α -particles (^4He) from decay of Th and U. Based on the major-element composition of Kula alkali basalt¹⁰, and assuming a K/U ratio of 10^4 , this sample should have a U concentration of ~ 0.8 p.p.m. Assuming Th/U of ~ 3 , $\sim 5 \times 10^{-8} \text{ cm}^3$ radiogenic $^4\text{He g}^{-1}$ would be produced in 500,000 yr, a minimum age for the Kula formation^{8,9}. Therefore, implantation of a very small fraction ($< 1\%$) of the matrix radiogenic helium could account for the low-temperature release of ^4He .

The simplest interpretation of Figs 1 and 2 is that the low to intermediate temperatures release the more weakly bound radiogenic and cosmogenic components, and high temperatures release primarily the inherited magmatic component. This explanation is consistent with both the crushing results and the heating patterns in Figs 1 and 2. Most of the helium in the phenocrysts (that contained in melt and spinel inclusions) is released by crushing, and is presumed to be representative of the inherited magmatic component, having a $^3\text{He}/^4\text{He}$ ratio of 8 R_a . Because most of the inherited helium is released by crushing, the powder remaining after crushing is most affected by additions of post-eruptive radiogenic and cosmogenic helium. Therefore, heating of olivine powder from sample HS85-3 released the highest $^3\text{He}/^4\text{He}$ ratio, up to 2,600 times atmospheric. The extremely high $^3\text{He}/^4\text{He}$ released by the low-temperature heating experiments on HS85-3 is most readily explained by the presence of cosmogenic ^3He .

There are several possible mechanisms for producing cosmogenic helium enriched in ^3He : the two most important are capture of thermal neutrons by ^6Li through $^6\text{Li}(n, \alpha)\text{T} \rightarrow ^3\text{He}$ and spallation reactions on all heavy nuclei to produce T and ^3He . The ^6Li reaction and spallation reactions produce $^3\text{He}/^4\text{He}$ of 1 and ~ 0.1 (roughly 10^6 and 10^5 times atmospheric), respectively. The ^3He production rate by the ^6Li reaction can be readily estimated once the thermal neutron flux is known. The region just below the atmospheric boundary layer will have a higher thermal neutron density due to thermalization of atmospheric fast neutrons^{16,17}. Therefore, because of the uncertain sampling depth, a minimum neutron density is given by the production rate within the basalt. The neutron production rate in water at 3 km elevation is roughly $1.3 \times 10^{-4} \text{ neutrons g}^{-1} \text{ s}^{-1}$ (ref. 18). Scaling according to atomic weight¹⁸, the neutron production in basalt should be roughly 25% higher than in water. Therefore, a minimum thermal neutron density in the basalt sample is $\sim 1.6 \times 10^{-4} \text{ neutrons g}^{-1} \text{ s}^{-1}$, but could be up to five times this value due to thermalization of fast neutrons, depending on the depth below the interface^{16,17}.

The fraction of thermal neutrons absorbed by ^6Li is given by $f = \sigma_{\text{Li}} N_{\text{Li}} / \sum \sigma_i N_i$ where σ is the neutron absorption cross-section (barns, b), and N is the number of moles of each element (i). Using the major- and trace-element composition of Kula alkali basalt¹⁰ for HS85-3, $\sum \sigma_i N_i = 0.0125 \text{ mol b g}^{-1}$. Assuming that the Li content is 10 p.p.m. (refs 19, 20), $f \approx 8.2 \times 10^{-3}$, and the ^3He production rate is between 40 and 200 atoms $\text{g}^{-1} \text{ yr}^{-1}$. Using the minimum age of the Kula formation ($\sim 500,000$ yr), this rate would produce between 7.5×10^{-13} and $3.7 \times 10^{-12} \text{ cm}^3 \text{ STP } ^3\text{He g}^{-1}$. Obviously, the depth and age are important unknowns in this calculation, but the ^6Li neutron mechanism alone could readily account for the observed ^3He concentrations ($\sim 1 \times 10^{-12} \text{ cm}^3 \text{ STP } ^3\text{He g}^{-1}$) even if the Li content were a factor of two lower.

The spallation production rate for ^3He can be calculated using the estimates of Yokoyama *et al.*²¹ for tritium. Assuming that the ^3He /tritium production ratio is ~ 1 (ref. 22), and scaling to the atmospheric depth and geomagnetic latitude of Haleakala, the ^3He production rate at the surface is $\sim 1,100 \text{ atoms g}^{-1} \text{ yr}^{-1}$. Assuming an attenuation scale of 180 g cm^{-2} (M.D.K. *et al.*, in preparation), the production rate at 50 cm would be $630 \text{ atoms g}^{-1} \text{ yr}^{-1}$ (using a density of 2 g cm^{-3}). These estimates would

Table 2 Stepwise heating experiments

Sample	Temperature (°C)	^3He (cm ³ STP)	Fraction of total ^3He	^4He (cm ³ STP)	Fraction of total ^4He	$^3\text{He}/^4\text{He}$ (R/R_a)
HS85-3						
Olivine powder (0.1814 g)	(550)	1.17×10^{-13}	0.613	1.18×10^{-10}	0.358	714 ± 12
	850	5.65×10^{-14}	0.296	1.56×10^{-11}	0.047	$2,600 \pm 235$
	1,000	1.09×10^{-14}	0.057	1.98×10^{-11}	0.060	400 ± 31
	1,170	3.50×10^{-15}	0.018	6.45×10^{-11}	0.195	39 ± 2
	1,350	2.33×10^{-15}	0.012	9.66×10^{-11}	0.293	17 ± 2
	1,750	6.99×10^{-16}	0.004	1.56×10^{-11}	0.047	32 ± 3
	Total	1.91×10^{-13} $1.05 \times 10^{-12*}$		3.30×10^{-10} $1.82 \times 10^{-9*}$		418
1-2 mm olivine (0.1233 g)	850	6.90×10^{-14}	0.409	1.83×10^{-10}	0.103	272 ± 3
	1,350	7.64×10^{-14}	0.453	7.47×10^{-10}	0.420	73.9 ± 3
	1,350	9.00×10^{-15}	0.053	2.29×10^{-10}	0.129	28.4 ± 1.0
	1,350	1.83×10^{-15}	0.011	4.90×10^{-11}	0.027	27 ± 2.5
	1,750	1.24×10^{-14}	0.074	5.72×10^{-10}	0.321	15.6 ± 3
	Total	1.69×10^{-13} $1.37 \times 10^{-12*}$		1.78×10^{-9} 1.44×10^{-8}		68.4
Clinopyroxene powder (0.1821 g)	(550)	7.75×10^{-14}	0.344	2.77×10^{-10}	0.368	202 ± 2
	850	1.39×10^{-13}	0.617	1.55×10^{-10}	0.206	649 ± 7
	850	3.58×10^{-15}	0.016	2.76×10^{-11}	0.037	94 ± 7
	1,700	5.15×10^{-15}	0.023	2.94×10^{-10}	0.390	12.7 ± 0.4
	Total	2.25×10^{-13} $1.23 \times 10^{-12*}$		7.54×10^{-10} $4.137 \times 10^{-9*}$		215
Matrix (0.1207 g)	(200)	1.07×10^{-15}	0.014	8.87×10^{-11}	0.044	8.7 ± 1.0
	(300)	3.57×10^{-14}	0.482	1.07×10^{-9}	0.534	24.1 ± 0.3
	(500)	3.42×10^{-14}	0.461	7.85×10^{-10}	0.391	31.5 ± 0.4
	900	2.07×10^{-15}	0.028	3.25×10^{-11}	0.016	46 ± 3
	1,250	1.09×10^{-15}	0.015	2.92×10^{-11}	0.015	27 ± 4
	Total	7.41×10^{-14} $6.14 \times 10^{-13*}$		2.00×10^{-9} 1.66×10^{-8}		26.7
H85-10						
Olivine powder (0.2128 g)	850	1.23×10^{-15}	0.160	5.52×10^{-10}	0.486	1.61 ± 0.21
	1,150	9.41×10^{-16}	0.122	1.01×10^{-10}	0.089	6.7 ± 1.0
	1,350	4.54×10^{-15}	0.591	3.96×10^{-10}	0.349	8.3 ± 0.3
	1,750	9.75×10^{-16}	0.127	8.68×10^{-11}	0.076	8.1 ± 1.5
	Total	7.69×10^{-15} $3.62 \times 10^{-14*}$		1.14×10^{-9} $5.34 \times 10^{-9*}$		4.89

Temperatures estimated by optical pyrometry; those in parentheses are estimated to be uncertain by 75 °C; all others uncertain by 30 °C. Each temperature step has been corrected for blank ^4He of $5.2 \pm 0.2 \times 10^{-11}$ cm³ STP and ^3He of 7.0×10^{-17} cm³ STP.

* cm³ STP g⁻¹.

correspond to 1.1×10^{-11} – 2.0×10^{-11} cm³ STP ^3He g⁻¹ produced in 500,000 yr, which is significantly greater than both the ^6Li production rate and the observed excess ^3He . Using the calculation method of Reedy and Arnold²³ yields a slightly lower production rate, but is within the uncertainties inherent in the elemental excitation functions and cosmic-ray energy spectrum (details will be given elsewhere).

The other production mechanisms are relatively unimportant for the upper several metres of basalt. The maximum production rate from muon capture processes (see ref. 5) can be estimated by taking the total muon stopping rate²⁴, and assuming that the total number of Li atoms reacting is $[N_{\text{Li}}/N_{\text{total}}]$; this mechanism is too low (by a factor of $\sim 10^5$) to be relevant. Takagi⁵ has estimated the rates for muon-induced photonuclear processes. Using his ^3He production rate, and a muon flux of $0.01 \text{ cm}^{-2} \text{ s}^{-1}$ (ref. 25), photonuclear processes are also unimportant to the present situation.

Based on these estimates, spallation is the dominant mechanism of cosmogenic ^3He production, although a preliminary report of this work attributed it to the ^6Li reaction²⁶. There are several important uncertainties in applying these production rates to sample HS85-3. Both the age and the sampling depth are only known approximately, which can clearly have a large

effect. Also, erosion may have affected the top of the lava flow. The thickness of this lava flow where it was sampled (1.5 m) is quite similar to the thickness at horizons that were later buried⁶, suggesting that erosion has been less than several metres, presumably because of effective drainage near the summit; low erosion rates are also indicated by the presence of intact cinder cones of Kula age⁶. More quantitative discussion of these variables awaits more detailed sampling. Nevertheless, the production rate calculations conclusively show that cosmogenic helium can easily account for the observed excess ^3He .

An additional uncertainty relates to the retention of the cosmogenic helium. The stepwise heating of the phenocrysts released significantly more ^3He than did heating of the basalt matrix. However, the matrix is clearly more susceptible to loss mechanisms because of the smaller grain size of roughly 50 μm , relative to the size of the phenocrysts, which are of the order of several millimetres. For example, if the cosmogenic production rate in the matrix has been similar to that in the phenocrysts, $\sim 40\%$ has been lost from the matrix. Note also that olivine and clinopyroxene phenocrysts from sample HS85-3 contain roughly the same quantity of cosmogenic ^3He ($\sim 1.1 \times 10^{-12}$ cm³ STP g⁻¹; Table 1). This could be explained by the fact that production by spallation is only weakly dependent on composition, with

virtually all ^3He produced by the major-element targets in the rock (that is, O, Si, Mg, Al). If the ^6Li reaction were the major production mechanism, this uniformity would require that either clinopyroxene and olivine contained equal concentrations of Li, or all the cosmogenic ^3He in the phenocrysts was implanted (as tritium) by the $^6\text{Li}(n, \alpha)\text{T}$ reactions in the matrix.

Extremely high $^3\text{He}/^4\text{He}$ ratios in Kula formation basalt are probably the result of cosmogenic helium, rather than primordial trapped helium^{12,13}. Cosmic-ray production rates, exposure ages, and stepwise-heating helium release patterns discussed here are all consistent with this hypothesis. Furthermore, the extremely high $^3\text{He}/^4\text{He}$ ratios are not observed in two 'shielded' samples of the same age, or in a historic lava flow (Table 1). A consistent stratigraphical $^3\text{He}/^4\text{He}$ trend for Haleakala has been documented²⁶ that strongly supports the notion that the surface sample contains cosmogenic helium. A more extensive series of samples, spanning the entire range of available ages from Haleakala, yield systematic variability with volcano evolution²⁶; all the shielded Hana and Kula series samples have $^3\text{He}/^4\text{He}$ ratios indistinguishable from MORB ($8 R_a$).

Cosmogenic helium has been neglected previously, and is clearly important for interpreting helium isotopic results from rock samples that have remained near the surface for long periods of time. In order for cosmogenic helium to be significant, the surface exposure time must be long enough and the initial trapped helium content must be low, factors that must be carefully considered before high $^3\text{He}/^4\text{He}$ ratios are attributed to 'primordial' helium. Because the cosmogenic $^3\text{He}/^4\text{He}$ ratio is so high, a very small contribution can greatly elevate the measured $^3\text{He}/^4\text{He}$ ratio. If some of the uncertainties regarding ^3He production rates and potential loss mechanisms can be resolved, several important geophysical applications will be possible. Cosmogenic nuclides could be used to study ancient cosmic-ray fluxes, terrestrial erosion rates (see refs 27, 28) and possibly exposure ages for terrestrial lava flows.

A result that also has important implications for helium isotope studies is the internal isotopic heterogeneity within olivine and clinopyroxene mineral separates. Although it has generally been assumed that phenocrysts in basaltic lavas reflect the helium isotopic composition of the host magma¹², the results reported here suggest that this is not necessarily true for older samples, even if cosmogenic helium is absent. Radiogenic helium will tend to lower the $^3\text{He}/^4\text{He}$ ratios obtained by total fusion. The combination of crushing in vacuum and stepwise heating techniques provide a valuable experimental protocol for addressing this problem.

I thank M. Garcia, F. Frey and J. Nicholls for providing the samples and for discussing the geology of Haleakala. W. J. Jenkins, R. Reedy and T. Trull provided useful comments and D. Lott and P. O'Brien, laboratory assistance. I also thank Molly Lumping for typing, and Marie Johnson and Colleen Hurter for literary assistance. This work was supported by grants NSF-0CE-83-15270 and NASA-ECG-NAG-9-69. This is Woods Hole contribution no. 6059.

Received 25 October 1985; accepted 22 January 1986.

1. Tolstikhin, I. N. in *Terrestrial Rare Gases* (eds Alexander, E. C. & Ozima, M.) (Japan Scientific Society Press, Tokyo, 1978).
2. Craig, H. & Lupton, J. in *The Sea* Vol. 7 (ed. Emiliani, C.) (Wiley, New York, 1981).
3. Bernatowicz, T. J. & Podosek, F. A. in *Terrestrial Rare Gases* (eds Alexander, E. C. & Ozima, M.) (Japan Scientific Society Press, Tokyo, 1978).
4. Jeffrey, P. M. & Hagan, P. J. *Nature* **223**, 1253 (1969).
5. Takagi, J. *Nature* **227**, 362-363 (1970).
6. Stearns, H. T. & MacDonald, G. A. *Hawaii Division Hydrol. Bull.* **7**, 1-344 (1942).
7. MacDonald, G. A. & Powers, H. A. *Bull. geol. Soc. Am.* **57**, 115-124 (1946).
8. McDougall, I. *Bull. geol. Soc. Am.* **75**, 107-128 (1964).
9. Naughton, J. J., MacDonald, G. A. & Greenberg, V. A. *J. Volcan. Geotherm. Res.* **7**, 339-335 (1980).
10. Chen, C. Y. & Frey, F. A. *Nature* **302**, 785-789 (1983).
11. Chen, C. Y. & Frey, F. A. *J. geophys. Res.* **90**, 8743-8768 (1985).
12. Kaneoka, I. & Takaoka, N. *Science* **208**, 1366-1368 (1980).
13. Rison, W. & Craig, H. *Earth planet. Sci. Lett.* **66**, 407-426 (1983).
14. Kurz, M. D., Meyer, P. & Sigurdsson, H. S. *Earth planet. Sci. Lett.* **74**, 291-305 (1985).

15. Kurz, M. D., Jenkins, W. J., Hart, S. R. & Clague, D. *Earth planet. Sci. Lett.* **66**, 388-406 (1983).
16. Bethe, H., Korff, S. A. & Glazek, G. *Phys. Rev.* **57**, 573-587 (1940).
17. Swetnick, M. J. *Phys. Rev.* **95**, 793-796 (1954).
18. Yamahita, M., Stephens, L. D. & Patterson, H. W. *J. geophys. Res.* **71**, 3817-3834 (1966).
19. Shaw, D. M., Vatin-Perignon, N. & Muysson, J. R. *Geochim. cosmochim. Acta* **41**, 1601-1607 (1977).
20. Bailey, J. C. & Gwóźdz, R. *Lithos* **11**, 73-84 (1978).
21. Yokoyama, Y., Reys, J.-L. & Guichard, F. *Earth Planet. Sci. Lett.* **36**, 44-50 (1977).
22. Kruger, S. T. & Heymann, D. *J. geophys. Res.* **73**, 4784-4787 (1968).
23. Reedy, R. C. & Arnold, J. R. *J. geophys. Res.* **77**, 537-555 (1972).
24. Hampel, W. & Kirsten, T. *Proc. 14th int. Cosmic Ray Conf.* **14**, 1895-1899 (1975).
25. Rossi, B. *High Energy Particles* (Prentice-Hall, New Jersey, 1952).
26. Kurz, M. D., O'Brien, P. A., Garcia, M. O. & Frey, F. A. *Eos* **66**, 1120 (1985).
27. Jha, R. & Lal, D. *Proc. 2nd Symp. on Natural Radiation Environment* (eds Vohra, K. G. et al.) 629-635 (Wiley Eastern Limited, New Delhi, 1981).
28. Lal, D. & Arnold, J. R. *Proc. Ind. Acad. Sci.* **94**, 1-5 (1985).

Small non-overlapping offsets of the East Pacific Rise

Rodey Batiza & Steven H. Margolis

McDonnell Center for the Space Sciences, Washington University, St Louis, Missouri 63130, USA

High-resolution bathymetric surveys^{1,2} of the East Pacific Rise (EPR) reveal that in addition to overlapping spreading centres (OSCs), the crest of the EPR is offset tiny amounts by smaller features. Recently, Langmuir *et al.* have shown that despite their subtle bathymetric expression, these small offsets, bends and kinks of the EPR, collectively termed deviations from axial linearity (devals), represent important petrological and tectonic boundaries. Some devals are small non-overlapping offsets (SNOOs) that offset the EPR axis by up to a few hundred metres. Bathymetric contours near SNOOs are usually disturbed from their axis-parallel trend in ways that we interpret as evidence for strike-slip faulting and volcanism at high angles to the trend of EPR axis. We offer a model for the formation of SNOOs and their associated faults that invokes independent magma supply, stochastic active spreading and limited non-rigid behaviour of the neovolcanic zone.

Macdonald *et al.*¹ and Lonsdale^{2,4} mapped OSCs at the EPR and recognized that bends, kinks and small offsets occur along the EPR axis. Langmuir *et al.*³ called attention to these smaller features, or devals, because in some cases they represent important petrological boundaries which, along with transform faults and OSCs, define the fundamental petrological and tectonic segmentation of the EPR. Small non-overlapping offsets (SNOOs) are devals which exhibit tiny (up to several hundred metres) lateral offsets of the EPR axis. Unlike OSCs, SNOOs exhibit no overlap. Previously unrecognized is the fact that near SNOOs, axis-parallel depth contours are commonly deflected to be perpendicular, or nearly so, to the EPR axis. We interpret these ridge-normal contour patterns as evidence for strike-slip faults and associated volcanism in the vicinity of SNOOs.

Figure 1 shows an example of a SNOO of the EPR near 13°45' N mapped by Macdonald *et al.*¹. It is a tiny offset of the EPR that occurs at a local bathymetric low point along the axis. On the south-west side of the SNOO, depth contours swing to the west and are nearly perpendicular to the ridge for several hundred metres away from the ridge crest. Other examples of SNOOs along the EPR can be seen in the published maps of Macdonald *et al.*¹ at 8°42', 9°00', 9°02', 9°04', 9°06', 10°20', 10°31', 10°42', 11°16', 11°45', 11°58', 12°10', 12°17', 12°27', 12°34', 12°36', 12°39', 12°50', 13°46', 13°47', 13°57', and 14°12'. Lonsdale² shows one at 3°48' N and another possible example is present at the Galapagos spreading centre at 86°05' W⁵. Particularly good examples, also known to be petrological boundaries³, are at 11°16', 12°10', 12°17', 12°27' and 13°47' (Fig. 1). These features are commonly: (1) tiny offsets of the ridge axis; (2) located at

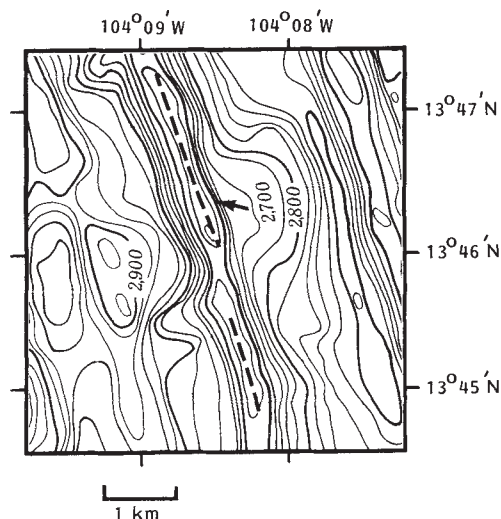


Fig. 1 Seabeam bathymetry (contour interval 20 m) from ref. 1 showing a small non-overlapping offset (SNOO) at 13°45' N. Note that on the west side of the ridge axis, depth contours veer away and become nearly perpendicular to the ridge. Although subtle bathymetric features like this are found along the ridge at many localities, they are a characteristic feature of SNOOs.

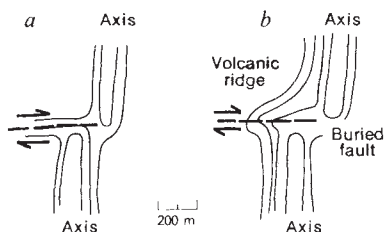


Fig. 2 Schematic drawing of SNOOs showing our interpretation of the subtle bathymetric disturbances that occur near them. In *a*, the model depth contours (1–5 m contour interval) define a strike-slip fault, expected to have only subtle bathymetric expression. In *b*, the bathymetric expression of the buried strike-slip fault is accentuated by the formation of a volcanic ridge. Both types of SNOO can be seen along the EPR^{1,2}. See text for discussion

a bathymetric low point along the axis; and (3) associated with disturbed, ridge-normal depth contours usually on only one side of the ridge. As shown in Fig. 2, we interpret the disturbed contours to represent ridge-normal strike-slip faults and volcanic ridges that, in some cases, bury them. Small submarine strike-slip features without dip-slip movement or volcanism should have only subtle bathymetric expression.

To explain the occurrence of SNOOs and their off-axis disturbed zones (presumed strike-slip faults) we offer a simple model for possible ridge crest behaviour that results in the formation of new SNOOs along previously continuous ridge crest segments. Since SNOOs can be major petrological boundaries, their origin may be linked to magma supply processes^{1,3}. Consequently, in our model we assume that the EPR is supplied by discrete magma systems along its length, for which there is a great deal of independent evidence^{1,3,6–8}. We also assume that spreading is stochastic. This means that each distinct tectonic and volcanic process associated with plate separation, such as fissuring, extensional normal faulting, dyke injection and deeper magma intrusion is episodic and its rate of occurrence is normally distributed about a mean value⁹. It is not yet known to what extent these distinct tectonic and volcanic processes are correlated, periodic or causally related^{10–18}, hence our model is

quite generalized. Finally, we assume that dyke injection or other intrusive processes cause lateral stresses near the surface¹⁹ sufficient to deform the upper parts of the crust. That is, we assume a causal relationship between igneous and tectonic components of spreading. This is speculative, but it is known that intrusion and surface deformation are correlated in some active volcanic rift zones^{20,21}.

According to this model, we view the spreading behaviour of adjacent but independently supplied ridge segments as uncorrelated. If a segment actively spreads while adjoining segments do not, offsets between segments will be created in the weak neovolcanic zone. These offsets behave as small ephemeral transform faults except that non-rigid plate deformation is required, which results in relative motion and strike-slip deformation outside the axial rift zone. Consider an initially straight ridge segment as in Fig. 3. Each independently supplied segment has an instantaneous spreading rate S_i , given by

$$S_i = \bar{S}_i + \sigma_i X \quad (1)$$

where $i = 1, 2$ for the two segments, $\bar{S}_i$ and σ_i are the mean and standard deviation of the spreading rate distribution and X is a standardized normal variable, a random variable with zero mean and unit variance. For simplicity, we assume that the mean spreading rate and standard deviation of each segment are equal (Fig. 3). The instantaneous spreading rate is simply equal to the mean rate plus (or minus) a randomly varying amount. Since each ridge segment selects instantaneous spreading rates episodically (with characteristic time τ) from a distinct, albeit identical, gaussian distribution, offsets between adjoining ridge segments will statistically appear and disappear if the model assumptions are met. The lengthening of these offsets is itself stochastic:

$$S_1 - S_2 = (\bar{S}_1 - \bar{S}_2) + \sqrt{(\sigma_1^2 + \sigma_2^2)} X \quad (2)$$

which can be rewritten

$$S_1 - S_2 - (\bar{S}_1 - \bar{S}_2) = \sigma X \quad (3)$$

where

$$\sigma^2 = (\sigma_1^2 + \sigma_2^2) \quad (4)$$

Next, we use a random walk model^{22,23} to consider the distribution of offset lengths after time t . This is:

$$P(x) = \frac{e^{-\frac{1}{2}(x/\sigma_R)^2}}{\sqrt{2\pi}\sigma_R} \quad (5)$$

where

$$\sigma_R = \sigma\sqrt{t\tau} \quad (6)$$

is the standard deviation of the distribution of offset lengths at time $t \gg \tau$. The probability of finding an offset larger than L at time t is the integral of this distribution:

$$P(x > L) = 2 \int_L^\infty P(x) dx \quad (7)$$

$$= \sqrt{\frac{2}{\pi}} \int_{L/\sigma_R}^\infty e^{-x^2/2} dx \quad (8)$$

Figure 4 shows the probability of occurrence of offsets of various lengths for different values of σ_R . For the EPR, if we choose a mean spreading rate of 6 ± 0.7 cm yr⁻¹ and $\tau = 10^4$ yr, then σ_R is 316 m for $t = 10^5$ yr. In this example, offsets on the order of 300 m would be expected to develop during a period over which about 6 km of total spreading occurred. Other values may be chosen, since actual values of σ and τ for the EPR are not known; however, the values chosen in this example do not seem unreasonable. This model thus shows that observed offsets at SNOOs ($\sim 10^2$ m in length) can be accounted for by the sort of model we propose.

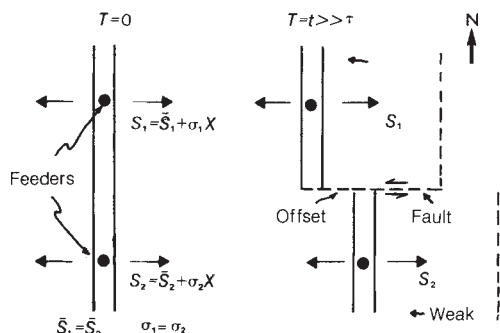


Fig. 3 Illustration of our proposed model for the origin of SNOO and ridge-normal structures. At time $T=0$, the initially straight ridge segment is fed by two independent feeders and the ridge portions associated with each feeder spread at instantaneous rates of S_1 and S_2 respectively. The mean spreading rates ($\bar{S}_1$ and $\bar{S}_2$) and the normally-distributed standard deviations from the mean (σ_1 and σ_2) are equal. In these idealized conditions, offsets will develop by deformation of the weak zone near the ridge axis due to tiny stochastic differences in S_1 and S_2 that accumulate after a time interval t . Although the mean and standard deviation of instantaneous spreading are the same for both ridges, the random component is selected from distinct, albeit identical, distributions. In this example we assume that spreading of segment 2 is fully asymmetric (east flanks weaker than west flank). If symmetric spreading occurred (east and west ridge flanks equally strong) no offset would develop, but strike-slip deformation would occur on both flanks.

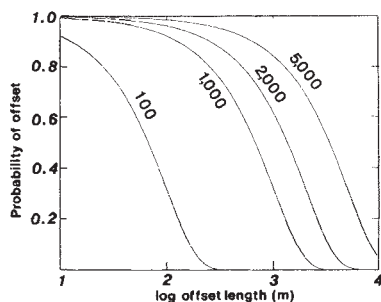


Fig. 4 Plot of expected offset length between two independently-fed ridge segments ($\log_{10}$ offset length in metres) versus the probability that it will be observed for several values of σ_R . σ_R is a function of the standard deviation of the mean spreading rate, the duration of spreading (t) and τ , the characteristic time of the spreading process. Offsets of several hundred metres can develop for $t \geq 10^5$ yr and reasonable choices of the other variables. See text for discussion.

As shown in Fig. 3, an episodic increment of spreading (intrusion and extension) at a ridge segment while the adjoining segment is quiet can be accommodated in two extreme ways. If both flanks of the rift are equally strong, the relative motion between two adjoining segments could be taken up by strike-slip deformation of both flanks. In this case, no offset between the ridge segments would be created. On the other hand, if one flank is weaker than the other because of local heterogeneity or some other reason, the necessary strike-slip deformation could be confined to one flank. Such behaviour could result in asymmetric spreading as in zero-offset transform faults²⁴.

According to our model, SNOOs are newly created as a direct consequence of independent magma supply systems along the EPR. In this regard, our model can be viewed as an extension of the model proposed by Macdonald *et al.*¹ for the behaviour of the EPR. The model has several predictions that can be tested when sufficient data of high resolution are available.

For example, the sea floor in the vicinity of SNOOs should exhibit evidence of strike-slip faulting and/or volcanic eruption along ridge-normal conduits. If evidence of SNOOs and their associated faults is preserved on older ocean floor away from the axis, their distribution could be used to infer the past geometry of magma feeders at the EPR and their temporal stability.

This study was supported by NSF and ONR. We thank the following persons for providing helpful comments on an earlier draft of this paper and for useful discussions: C. Langmuir, J. Bender, K. Macdonald, J. Fox, H. Schouten, R. Hey, P. Lonsdale, J. Allan and D. Wiens. We thank K. Macdonald and J. Fox for kindly providing copies of charts of the EPR.

Received 19 August 1985; accepted 9 January 1986.

1. Macdonald, K. C., Sempere, J.-C. & Fox, P. J. *J. geophys. Res.* **89**, 6049–6069 (1984).
2. Lonsdale, P. *Bull. geol. Soc. Am.* **96**, 313–327 (1985).
3. Langmuir, C. H., Bender, J. F. & Batiza, R. (in preparation).
4. Lonsdale, P. *J. geophys. Res.* **88**, 9393–9406 (1983).
5. Ballard, R. D., Van Andel, T. H. & Holcomb, R. T. *J. geophys. Res.* **87**, 1149–1161 (1982).
6. Whitehead, J. A., Dick, H. J. B. & Schouten, H. *Nature* **312**, 146–148 (1984).
7. Schouten, H., Klitgord, K. D. & Whitehead, J. A. *Nature* **317**, 225–229 (1985).
8. Crane, K. *Earth planet. Sci. Lett.* **72**, 405–414 (1985).
9. Schouten, H. and Denham, C. R. in *Deep Drilling Results in the Atlantic Ocean: Ocean Crust* (eds Talwani, M., Harrison, C. G. & Hayes, D. E.) 151–159 (American Geophysical Union, Washington DC, 1979).
10. Atwater, T. in *Deep Drilling Results in the Atlantic Ocean: Ocean Crust* (eds Talwani, M., Harrison, C. G. & Hayes, D. E.) 33–42 (American Geophysical Union, Washington DC, 1979).
11. Lister, C. R. B. *EOS* **63**, 1153 (1982).
12. Deffeyes, K. S. in *Megatectonics of Continents and Oceans* (eds Johnson, H. & Smith, B. L.) 194–222 (Rutgers University Press, New Brunswick, 1970).
13. Anderson, R. N. & Nolltimier, H. C. *Geophys. J. R. astr. Soc.* **34**, 137–147 (1973).
14. Lachenbruch, A. H. *J. geophys. Res.* **78**, 3395–3417 (1973).
15. Lachenbruch, A. H. *J. geophys. Res.* **81**, 1883–1902 (1976).
16. Tappinier, P. & Francheteau, J. *J. geophys. Res.* **83**, 3995–3970 (1978).
17. Sleep, N. in *Hydrothermal Processes at Seafloor Spreading Centers* (eds Rona, P. A., Bostram, K., Laubier, L. & Smith, K. L.) 71–82 (Plenum, New York, 1983).
18. Kappel, E. S. & Ryan, W. B. F. *EOS* **65**, 1110 (1984).
19. Spence, D. A. and Turcotte, D. L. *J. geophys. Res.* **90**, 375–580 (1985).
20. Bjornsson, A., Sigurdsson, S. & Thorbergsson, G. *J. geophys. Res.* **84**, 3029–3028 (1979).
21. Duffield, W. A., Jackson, D. B. & Swanson, D. A. in *Proc. Symp. Andean and Antarctic Volcanology Problems* 577–587 (Giannini and Figli, Napoli, 1976).
22. Feller, W. *An Introduction to Probability Theory and its Applications*, Vol. I (John Wiley, New York, 1968).
23. Uhlenbeck, G. E. & Ornstein, L. S. *Phys. Rev.* **36**, 823 (1930).
24. Schouten, H. & White, R. S. *Geology* **8**, 175–179 (1980).

The last pluvial climatic episodes in the deserts of southwestern North America

W. Geoffrey Spaulding* & Lisa J. Graumlich†

* Quaternary Research Center and Department of Botany, University of Washington, Seattle, Washington 98195, USA

† Department of Ecology and Behavioral Biology, University of Minnesota, Minneapolis, Minnesota 55455, USA

Long-term variations in the effective moisture of deserts are linked both to glacial–interglacial climatic cycles and, through the Milankovitch theory, to orbitally induced changes in solar radiation intensity^{1–4}. For southwestern North America, comparison of palaeobotanical data with model predictions of palaeoclimate reveals two distinct pluvial regimes during the late Wisconsin and early Holocene (23–8 kyr BP). Southward displacement of the Aleutian low-pressure centre and strengthened westerlies from ~40° to 30° N were important features of the full-glacial (~18 kyr BP) pluvial maximum. Increased cyclonic storms and weakened monsoons led to a winter precipitation regime, a feature seen both in the fossil record^{5,6} and climate models^{7,8}. Dominantly zonal flow during this period contrasts with the latest Wisconsin and early Holocene (12–8 kyr BP), when meridional circulation transported maritime tropical air into the desert interior. We now show

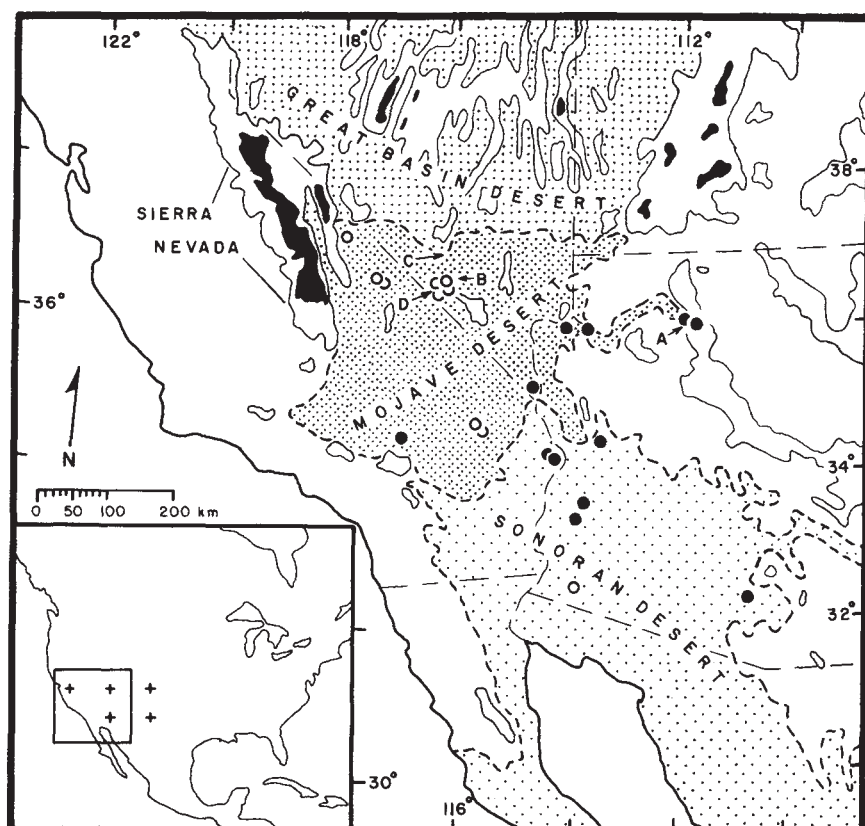


Fig. 1 The Sonoran, Mojave and southern Great Basin Deserts of North America. Solid lines indicate the 2,000-m contour interval; topography above 3,000 m is shaded. Packrat midden records from below 1,200 m elevation dating from 12 to 8 kyr BP are of desert scrub (open circles) and woodland (closed circles). Other localities mentioned in the text are: A, Grand Canyon; B, Skeleton Hills and Point of Rocks; C, Eleana Range; D, Amargosa Desert. Inset shows the grid points (+) in the Community Climate Model from which area averages were derived for the south-west (Fig. 2).

that plant macrofossil assemblages from packrat (*Neotoma* spp.) middens⁹ in the Mojave Desert provide evidence for increased temperatures and summer rainfall during this period¹⁰⁻¹². The anomalously late persistence (to ~8 kyr BP) of woodland in low-latitude, low-elevation deserts of the south-west¹³ is attributed to enhanced monsoonal circulation³.

North America's deserts range from the high-latitude steppe of the Great Basin to the subtropical scrub of the Sonoran Desert (Fig. 1). Precipitation seasonality varies: the Great Basin and Mojave Deserts receive <25% of annual precipitation during the summer, whereas the Sonoran Desert has a distinct summer monsoon which accounts for 40-60% of annual precipitation^{14,15}. During the full glacial the Sonoran Desert supported widespread woodland characterized by pygmy conifers (*Pinus monophylla*, *Juniperus* spp.), live oaks (*Quercus turbinella*, *Q. dumii*) and succulents (Agavaceae, Cactaceae). The composition of diverse woodland communities in Sonoran Desert lowlands, and their location more than 600 m below current woodland on nearby mountains, indicates increased annual precipitation (perhaps double today's amounts), reduced summer temperatures and mild winters^{5,6}.

North of ~36° N, woodland also occurred far below its current lower altitudinal limit. However, these full-glacial communities did not support the diversity of trees and succulents found farther south. Five low-elevation (<1,200 m), full-glacial Sonoran Desert middens^{16,17} provide records of 7 tree species, 14 succulents and 2 steppe shrubs. In contrast, nine low-elevation Mojave Desert sites^{10,18} yield only 3 tree species and 6 succulents, but 8 steppe shrub taxa, indicating an effectively drier palaeoclimate. Indeed, the full-glacial Mojave Desert was sufficiently dry to support desert vegetation below 1,000 m elevation^{18,19}. Zoned above desert scrub, xerophytic juniper woodland ranged to 1,800 m. Subalpine woodland occurred at higher elevations, dominated by cold-tolerant xerophytic conifers, limber pine (*Pinus flexilis*) and bristlecone pine (*P. longaeva*)^{20,21}. Steppe shrubs were widespread not only in desert vegetation, but also in low-elevation and subalpine woodlands⁶.

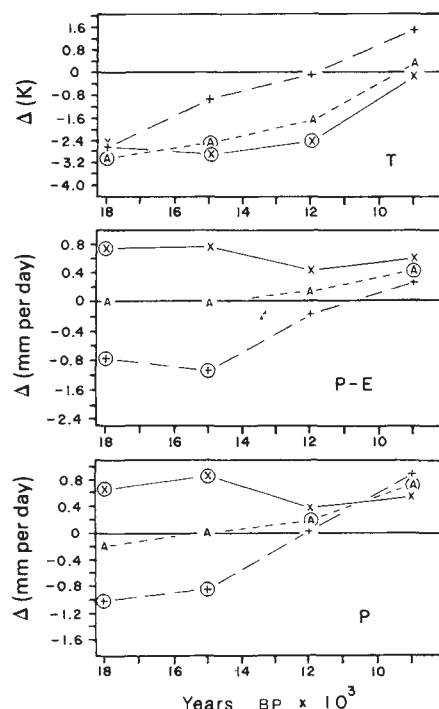


Fig. 2 Area averages for five land-surface grid points representing southwestern North America (Fig. 1) in the Community Climate Model, programmed for boundary conditions at 3-kyr intervals from 18 to 9 kyr BP. The zero value (horizontal line) in each case represents the result of the control experiment for modern conditions. Model results for temperature (*T*), precipitation minus evaporation (*P-E*), and precipitation (*P*) are expressed in terms of deviations (Δ) for simulations of January ($\times$), July ($+$) and average annual (*A*) conditions. Circled symbols represent significant differences from the control result at the 95% level (two-sided *t*-test).

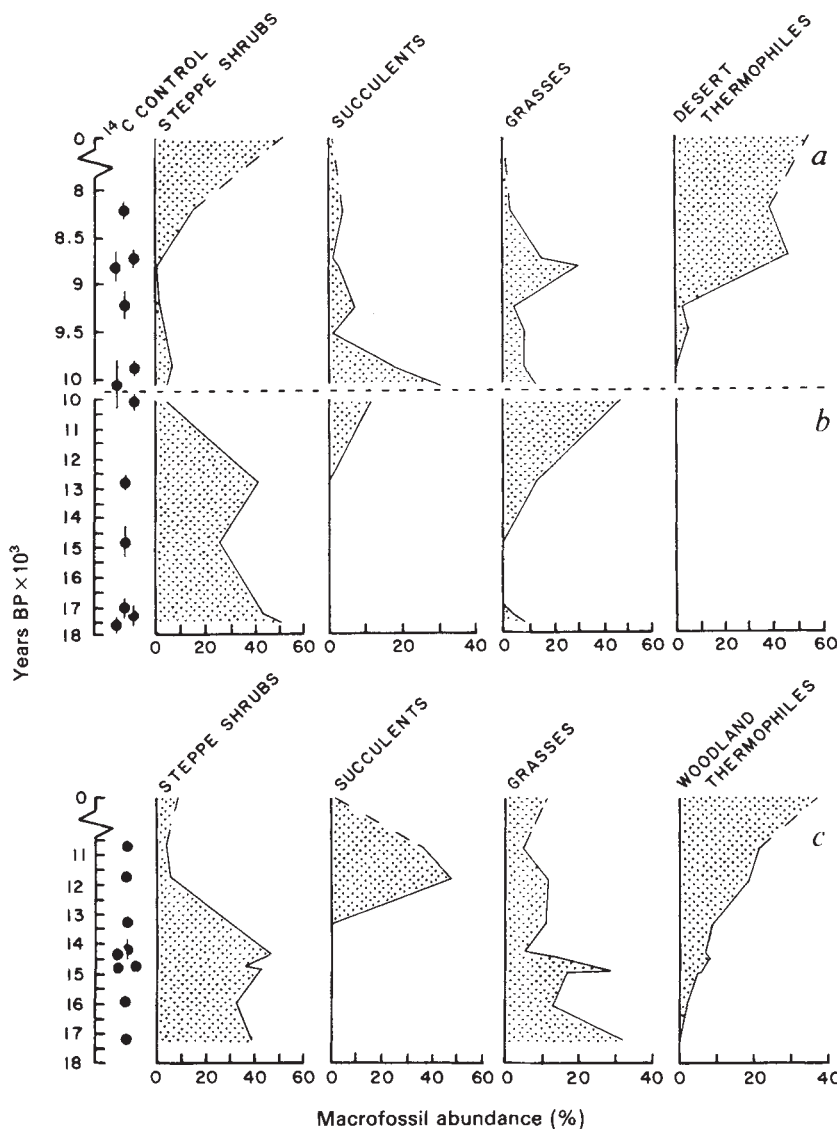


Fig. 3 Relative abundance of selected plant macrofossils from radiocarbon-dated assemblages collected at three packrat midden sites in the northern Mojave Desert. *a*, Skeleton Hills; *b*, Point of Rocks; *c*, Eleana Range. Macrofossil abundance expressed as percentages of the total number of identified specimens minus the number of specimens of the dominant taxon (NISP-DT). Dashed lines separate the youngest macrofossil assemblages at the Eleana Range-2 and Skeleton Hills-2 sites from the modern control samples collected at those sites. Note changes in the time scale.

Mojave Desert macrofossil assemblages reflect a glacial climate distinct from that of the Sonoran Desert. The absence of thermophiles suggests a decline in annual temperatures of $>6^{\circ}\text{C}$ (ref. 10), contrasting with an estimated decline of $<4^{\circ}\text{C}$ for the Sonoran Desert¹⁶. Full-glacial desert scrub and the xerophytic nature of Mojavean woodland accord with a 'pluvial' climate characterized by an increase in annual precipitation of not more than 40%^{10,12}. A survey of potential pluvial lake basins in Nevada confirms comparatively dry conditions; many closed valleys in the southern Great Basin have no shoreline features²².

The mean position of the winter storm track, now north of 42°N (ref. 23), was shifted south during the full glacial, accounting for pluvial climatic conditions in the south-west⁵⁻⁷. Simulations using the US National Center for Atmospheric Research Community Climate Model (CCM) replicate this circulation feature^{8,24}. Mesoscale heterogeneity is smoothed by area averages for the south-west (Fig. 2), but these show significant reductions in July precipitation and increases in January precipitation. The transition to relatively dry conditions north of 36°N was topographically controlled. The Sierra Nevada mountain range, reaching altitudes $>3,000\text{ m}$ north of 36°N (Fig. 1), is a barrier to westerly airflow. In its lee, conditions were relatively dry, while a pronounced pluvial occurred south of 36°N . Such regional variation of palaeoclimate calls into question any single reconstruction put forward to account for palaeophenomena of this subcontinental-scale region.

Decreasing effective moisture is evident by $\sim 15\text{ kyr BP}$ in the pluvial lake²⁵⁻²⁷ and packrat midden record¹². This late glacial climatic change accentuated regional vegetation differences. Between 10 and 8 kyr BP woodland persisted in the Sonoran Desert¹³ but had retreated from most low-elevation sites in the Mojave Desert²⁸, a marked anomaly considering that, 2-4° farther south, woodland occurred more than 400 m lower in elevation (Fig. 1). Where desertification might be expected earliest, at low elevations and latitudes in the Sonoran Desert, one finds instead the persistence of woodland long after its demise to the north. The distribution of low-elevation woodland from $\sim 12-8\text{ kyr BP}$ (Fig. 1) is congruent with the present pattern of summer rainfall dominance in the south-west^{23,29}.

Simulations of early Holocene climate^{2,8,30} and field evidence from Africa and India^{3,31-33} indicate strengthened monsoons at subtropical latitudes. In light of these studies and the following data, we propose that post-glacial woodland persistence in the Sonoran Desert was due to intensified monsoonal precipitation. Strengthened monsoons should be reflected in the fossil record from the summer-dry northern Mojave Desert by increases in succulents and grasses (Poaceae), plants that today are important in areas which receive high proportions of summer rains. Steppe shrubs, typical of winter-rainfall regimes, are expected to be abundant when winter rains dominate.

Assemblages from the Point of Rocks-3 (PR-3) and Eleana Range-2 (ER-2) sites record palaeovegetation in distinctly

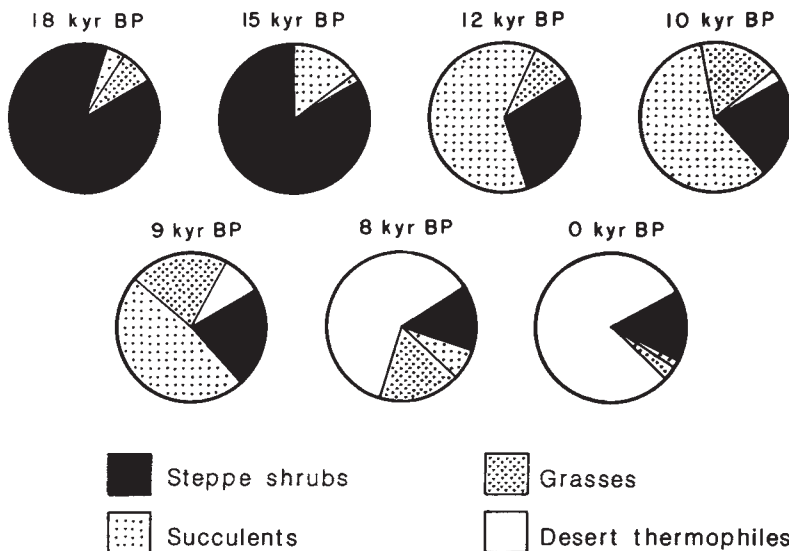


Fig. 4 Averaged relative abundance of selected plant types from macrofossil assemblages at sites below 1,200 m elevation in the Amargosa Desert, an intermontane valley in the northern Mojave Desert (Fig. 1). All packrat midden samples of an age within 1 kyr of the specified age class are considered in averages for that class. Number of sites, with number of samples in parentheses: 18 kyr, 4(6); 15 kyr, 2(3); 12 kyr, 2(5); 10 kyr, 8(14); 9 kyr, 5(12); 8 kyr, 1(3); 0 kyr, 9(9).

different habitats from ~17.5 to 10 kyr BP. PR-3 at 930 m elevation supports xerophytic Mojave Desert scrub, while ER-2 at 1,800 m is at the lower limit of modern woodland. During the full glacial ER-2 supported limber pine and steppe shrubs, while vegetation at PR-3 was dominated by steppe shrubs alone^{10,12}. Increasing woodland thermophiles at ER-2 after ~15 kyr BP (Fig. 3) is consistent with CCM-simulated increases in July temperature (Fig. 2). Steppe shrubs decline between 14 and 12 kyr BP (Fig. 3). Samples dating from ~12–10 kyr BP contain abundant succulents and, at the PR-3 site, grasses, indicating summer precipitation. At Skeleton Hills-2, a site similar to PR-3, succulents are important only in samples dated at ~10 kyr BP, suggesting that intensified summer rains ended shortly thereafter (Fig. 3). Steppe shrubs in the modern sample properly reflect winter seasonality of precipitation. Figure 4 summarizes macrofossil data from an intermontane valley in the northern Mojave Desert. Changes in steppe shrub, succulent and grass abundance are those expected from full-glacial winter precipitation dominance, followed by latest Wisconsin and early Holocene summer rainfall dominance.

CCM area averages (Fig. 2) show substantial relative increases in July precipitation during the latest Wisconsin and early Holocene, consistent with fossil data from the Mojave Desert and the Grand Canyon, and attributed to increased summer insolation. The last solar radiation maximum during northern-latitude summer occurred between 11 and 9 kyr BP³⁵. Enhanced monsoons also explain the persistence of woodland in the Sonoran Desert, closest to sources of maritime tropical moisture in the Gulf of Mexico and Pacific Ocean. Absence of summer-rain-dependent Sonoran Desert perennials has led to the assertion that monsoons were unimportant at this time¹³. Most Sonoran taxa are frost-sensitive and a CCM area-averaged January temperature for 12 kyr BP is ~2.4 °C below that of the control. The end of this period of relatively cold winter temperatures occurred by ~9 kyr BP (Fig. 2), when desert thermophiles began to increase (Figs 3, 4).

In conclusion, the deserts of southwestern North America lie at higher latitudes, and were in closer proximity to continental ice sheets than those of North Africa and India. Southward deflection of the Pacific westerlies led to a full-glacial pluvial climate. Topographic modulation of airflow created distinct subregional climates. South of the Sierra Nevada, precipitation was greatly enhanced during the full glacial, while in its lee, conditions were drier. Full-glacial CCM area averages do not simulate a decline in July temperature appreciably greater than that for January temperature (Fig. 2), a failure to replicate conditions called for in reconstructions stressing increased equability^{5,36}. This is consistent with recent studies indicating substantial lowering of both January and July temperatures^{10,12}.

Annual $P - E$ (precipitation minus evaporation) simulations for the full glacial approximate modern values (Fig. 2), and conflict with most data indicating increased effective moisture throughout the south-west. This may indicate a deficiency in the model, although results are sensitive to choice of representative grid points. Enhanced monsoons between ~12 and ~9 kyr BP had greatest impact in the Sonoran Desert, explaining delayed desertification there by the overlap of two distinct pluvial episodes. The first, associated with full-glacial climate dynamics, was characterized by lowered temperatures and enhanced winter rainfall. The second, induced by changes in seasonal insolation, was characterized by annual temperatures near the modern mean and enhanced monsoons.

This research was supported by the US Geological Survey, the US Department of Energy, and the NSF. We thank John Kutzbach for discussion and for CCM model results.

Received 15 October 1985; accepted 5 February 1986.

- Berger, A. L. *et al.* (eds) *Milankovitch and Climate* (Reidel, Dordrecht, 1984).
- Kutzbach, J. E. & Otto-Bleisner, B. L. *J. Atmos. Sci.* **39**, 1177–1188 (1982).
- Kutzbach, J. E. & Street-Perrott, F. A. *Nature* **317**, 130–134 (1985).
- Davis, O. K. *Science* **225**, 617–619.
- Van Devender, T. R. & Spaulding, W. G. *Science* **204**, 701–710 (1979).
- Spaulding, W. G., Leopold, E. B. & Van Devender, T. R. in *Late Quaternary Environments of the United States*, Vol. 1 (ed. Porter, S. C.) 259–293 (University of Minnesota Press, Minneapolis, 1983).
- Antevs, E. *Amer. Antiquity* **20**, 317–335 (1955).
- Kutzbach, J. E. & Guetter, P. J. *J. Atmos. Sci.* (in the press).
- Wells, P. V. *Quat. Res.* **6**, 223–248 (1976).
- Spaulding, W. G. *U.S. Geol. Surv. Prof. Pap.* 1329 (1985).
- Cole, K. L. *Science* **217**, 1142–1145 (1982).
- Spaulding, W. G., Robinson, S. W. & Paillet, F. L. *U.S. Geol. Surv. Water Resources Invest. Rpt.* 84-4328 (1984).
- Van Devender, T. R. *Science* **198**, 189–192 (1977).
- Hastings, J. R. & Turner, R. M. *The Changing Mile* (University of Arizona Press, Tucson, 1965).
- Mitchell, V. L. *J. appl. Met.* **15**, 920–927 (1976).
- Van Devender, T. R. thesis, Univ. Arizona, Tucson (1973).
- King, J. E. & Van Devender, T. R. *Quat. Res.* **8**, 191–204 (1977).
- Spaulding, W. G. *Curr. Res. Pleistocene* **2**, 83–85 (1985).
- Wells, P. V. & Woodcock, D. *Madrone* **32**, 11–23 (1985).
- Thompson, R. S. & Mead, J. I. *Quat. Res.* **17**, 39–55 (1982).
- Spaulding, W. G. in *Eighth North American Forest Biology Workshop* (ed. Lanner, R. M.) 42–69 (Utah State University, Logan, 1984).
- Miffin, M. D. & Wheat, M. M. *Nevada Bur. Mines Geol. Bull.* **94** (1979).
- Bryson, R. A. & Hare, F. K. in *Climates of North America* (eds Bryson, R. A. & Hare, F. K.) 1–48 (Elsevier, Amsterdam, 1974).
- Kutzbach, J. E. & Wright, H. E., Jr *Quat. Sci. Rev.* (in the press).
- Scott, W. E. *et al. Quat. Res.* **20**, 261–285 (1983).
- Thompson, R. S., Benson, L. V. & Hattori, E. M. *Quat. Res.* **25**, 1–9 (1986).
- Smith, G. I. *U.S. Geol. Surv. Prof. Pap.* 1043 (1979).
- Spaulding, W. G. *Quat. Res.* **19**, 256–264 (1983).
- Bryson, R. A. & Lowry, W. P. *Bull. Amer. met. Soc.* **36**, 329–339 (1955).
- Kutzbach, J. E. in *Late Quaternary Environments of the United States*, Vol. 2 (ed. Wright, H. E. Jr) 271–277 (University of Minnesota Press, Minneapolis, 1983).
- Bryson, R. A. & Swain, A. M. *Quat. Res.* **16**, 135–145 (1981).
- Talbot, M. R. *et al. in Palaeoecology of Africa* Vol. 16 (eds Coetzee, J. A. & Van Zinderen Bakker, E. M.) 173–192 (Balkema, Rotterdam, 1984).
- Richie, J. C., Eyles, C. H. & Haynes, C. V. *Nature* **314**, 352–355 (1985).
- Cole, K. L. *Am. Nat.* **125**, 289–303 (1985).
- Berger, A. L. *Quat. Res.* **9**, 139–167 (1978).
- Van Devender, T. R. & Wiseman, F. M. in *Paleoindian Lifeways* (ed. Johnson, E.) 13–27 (West Texas Museum Association, Lubbock, 1977).

Neuronal cell-cell adhesion depends on interactions of N-CAM with heparin-like molecules

Gregory J. Cole, Arleen Loewy & Luis Glaser

Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, 660 S. Euclid Avenue, St Louis, Missouri 63110, USA

Cell-cell interactions are of critical importance during neural development, particularly since the migration of neural cells and the establishment of functional interactions between growing axons and their target cells^{1,2} has been suggested to depend upon cell recognition processes. Neurone-neurone adhesion has been well studied *in vitro*, and is mediated in part by the neural cell adhesion molecule N-CAM³⁻⁷. N-CAM-mediated cell-cell adhesion has been postulated to occur by a homophilic binding mechanism⁸, in which N-CAM on the surface of one cell binds to N-CAM on a neighbouring cell. Studies in our laboratory have identified a cell surface glycoprotein, now known to be N-CAM⁹, which participates in cell-substratum interactions in the developing chicken nervous system¹⁰⁻¹². Although this adhesion involves a homophilic binding mechanism, the binding of the cell surface proteoglycan heparan sulphate to the glycoprotein is also required¹³. This raises the question of whether the binding of heparan sulphate to N-CAM is also required for cell-cell adhesion. Here we show that the binding of retinal probe cells to retinal cell monolayers is inhibited by heparin, a functional analogue of heparan sulphate, but not by chondroitin sulphate. Monoclonal antibodies that recognize two different domains on N-CAM, the homophilic-binding and heparin-binding domains, inhibit cell-cell adhesion. The heparin-binding domain isolated from N-CAM by selective proteolysis also inhibits cell-cell adhesion when bound to the probe cells.

The role of the heparin-binding domain of N-CAM in cell-cell adhesion was examined by measuring the binding of ³⁵S-methionine-labelled probe cells to monolayers of retinal cells, as described by Gottlieb *et al.*¹⁴. This assay system was selected as we find it gives more reproducible results than the cell aggregation assay. Embryonic day 11 retinal cells added to glass mini-vials derivatized with 3-aminopropyltriethoxysilane form dense retinal cell monolayers and the rate of adhesion of ³⁵S-methionine-labelled retinal probe cells to this layer can be quantitated. Labelled embryonic day 11 retinal probe cells adhere to the monolayers in a time-dependent manner, with approximately 70% binding after 1 h (Fig. 1).

To examine the effect of heparin (heparan sulphate) on cell-

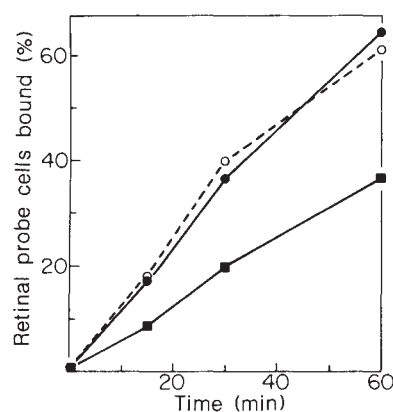


Fig. 1 Adhesion of metabolically labelled retinal probe cells to retinal cell monolayers. Open circles show the binding of untreated control cells to retinal cell monolayers. The labelled retinal cells bind to the monolayers in a time-dependent manner. Closed circles represent cell to cell adhesion in the presence of 100 µg ml⁻¹ chondroitin sulphate (Sigma, grade III mixed isomers), indicating that cell adhesion is not affected by chondroitin sulphate. Closed squares show the adhesion of retinal cells in the presence of 100 µg ml⁻¹ heparin (Sigma, No. H-3125). Adhesion is inhibited approximately 40% by heparin at all time points, and suggests that the inhibitory activity of heparin is specific.

Methods. Embryonic day 11 chicken retinas were dissociated by incubation for 15 min at 37 °C in 0.05% trypsin in calcium-magnesium-free Hank's solution. The cells were then triturated with a fire-polished Pasteur pipette and incubated overnight in Dulbecco's minimal essential medium containing 15% horse serum to allow recovery of cell surface proteins. Glass mini-vials (1.7 cm² surface area) were then derivatized with 3-aminopropyltriethoxysilane^{12,13} and 3 × 10⁶ recovered retinal cells were added to each vial¹⁴. Cells were coupled to the derivatized surface by centrifugation at 600g for 5 min, and the vials were then incubated at 37 °C for at least 1 h. Throughout the assay the retinal cells were maintained in Earle's balanced salts solution containing 0.2% albumin. To assay cell adhesion, mechanically dissociated embryonic day 11 retinal cells were pulse-labelled for 1-2 h with 10 µCi of ³⁵S-methionine (translation grade, NEN) and approximately 2 × 10⁵ cells (0.25 c.p.m. per cell) were added to each vial. Vials were then incubated at 37 °C on a gyratory shaker (100 r.p.m.). At the indicated times, the assay medium was aspirated and cell adhesion to the monolayer was quantitated by dissolving the cell in 1% Triton X-100, followed by liquid scintillation counting. To assess the effect of chondroitin sulphate or heparin on cell-cell adhesion, assays were conducted with 100 µg ml⁻¹ of the glycosaminoglycan present in the assay medium.

cell adhesion, cell adhesion assays were conducted in the presence of various concentrations of heparin or chondroitin sulphate. Heparin, a functional analogue of heparan sulphate, was used in these assays because it is more readily available from commercial suppliers. We have shown previously that cell-substratum adhesion is inhibited with similar efficacy by both heparin and the physiologically relevant ligand heparan sulphate. Chondroitin sulphate was used as a control to rule out any nonspecific inhibition resulting from the charge characteristics of the glycosaminoglycan.

Cell-cell adhesion is not affected by chondroitin sulphate (100 µg ml⁻¹), but is partially inhibited by a similar concentration of heparin (Fig. 1). The effect of various concentrations of the glycosaminoglycans on cell-cell adhesion is summarized in Table 1. This adhesion occurred at rates equivalent to control cultures at all concentrations of chondroitin sulphate tested. Increasing concentrations of heparin showed a concentration-dependent inhibition of cell-cell adhesion (Table 1). Only slight (20%) inhibition of cell to cell adhesion was observed with 50 µg ml⁻¹ of heparin, even though this concentration abolishes retinal cell attachment to N-CAM linked to a substrate^{9,13}, perhaps because a high quantity of cells was used in the cell-cell adhesion assay and 50 µg ml⁻¹ of heparin may not be sufficient

Table 1 Inhibition of cell-cell adhesion by heparin

Treatment	Probe cells bound (%)	Inhibition (%)
Control	63.5 ± 3.7 (6)	—
Chondroitin sulphate	62.0 ± 2.6 (5)	2.4
50 µg ml ⁻¹	64.1 ± 5.9 (2)	0
Heparin	51.9 ± 0.6 (2)	19.3
100 µg ml ⁻¹	37.0 ± 2.7 (5)	41.7
200 µg ml ⁻¹	22.0 ± 5.4 (3)	65.4

Cell-cell adhesion assays were conducted as described in Fig. 1 legend. ³⁵S-methionine-labelled retinal probe cells were incubated for 1 h at 37 °C with monolayers of retinal cells. The assay medium contained various concentrations of glycosaminoglycans as indicated. After incubation of labelled cells with monolayers, the assay medium was aspirated, cells were solubilized in 1% Triton X-100, and radioactivity quantitated by liquid scintillation counting. Data are expressed as mean ± s.d. For each experiment duplicate vials were assayed, and the number of independent experiments conducted is indicated in parentheses.

Table 2 Inhibition of cell-cell adhesion by anti-N-CAM monoclonal antibodies and heparin-binding domain of N-CAM

Treatment	Probe cells bound (%)	Inhibition (%)
Control	62.3 ± 5.1 (3)	—
25K heparin-binding N-CAM fragment	42.3 ± 4.4 (5)	32.1
C ₁ H ₃ monoclonal	20.2 ± 6.6 (3)	67.6
B ₁ A ₃ monoclonal in assay	18.4 ± 2.9 (2)	70.5
B ₁ A ₃ monoclonal, washed	29.8 ± 2.6 (2)	52.2

Cell-cell adhesion assays were conducted as described previously. To assess the effect of heparin-binding domain on cell-cell adhesion, aliquots of ³⁵S-methionine-labelled probe cells were incubated for 1 h at 4 °C with 50 µg ml⁻¹ of the heparin-binding domain. The domain was isolated by digesting immunopurified N-CAM with a 1:50 enzyme to substrate ratio of subtilisin protease⁹. The digested protein was then incubated with heparin-agarose, and the heparin-binding domain was eluted with 1 M NaCl⁹. Following incubation of the labelled probe cells with the domain, the cells were added to derivatized vials coated with retinal cells. The effect of anti-N-CAM monoclonals on cell-cell adhesion was determined by incubating the retinal cell monolayers with 0.5 mg ml⁻¹ of B₁A₃ Fab fragments or C₁H₃ monoclonal antibody for 1 h. The monolayers were then washed and labelled probe cells were added. Previous studies have also shown that B₁A₃ Fab fragments inhibit cell to substratum adhesion more efficiently when present in the assay medium. Therefore, assays were also conducted with 0.5 mg ml⁻¹ of B₁A₃ Fab fragments present in the assay medium. In the above experiments, control retinal cell monolayers were also incubated with 0.5 mg ml⁻¹ of control IgG. When monolayers were treated with anti-retinal cell monoclonal antibodies which do not affect cell-substratum adhesion, no inhibition was observed (data not shown).

to saturate the heparin-binding sites of N-CAM on both the monolayer and the probe cells. The inhibition of adhesion by 65% with 200 µg ml⁻¹ of heparin is particularly striking, since a similar concentration of chondroitin sulphate does not diminish the rate of cell-cell adhesion. These data suggest that adhesion between retinal cells depends upon cell surface heparan sulphate proteoglycan. It has been shown previously that an antiserum directed against this class of proteoglycan inhibits retinal cell-substratum adhesion^{9,13,15}. In view of our previous data demonstrating that N-CAM participates in retinal cell-substratum adhesion and that it possesses a heparin-binding domain^{9,13}, we postulate that the binding of cell surface heparan sulphate to N-CAM is required for cell-cell adhesion.

To determine if heparin inhibits N-CAM-mediated cell-cell adhesion, we used both the isolated heparin-binding domain of N-CAM and a monoclonal antibody that recognizes the heparin-binding domain⁹ to examine whether heparin-binding by N-CAM is required for cell adhesion. The C₁H₃ monoclonal antibody inhibits cell-substratum adhesion by a mechanism suggestive of the homophilic binding that has been demonstrated for N-CAM-mediated cell-cell adhesion⁸, but our previous studies implied that heparan sulphate binding to N-CAM was required for homophilic binding to occur¹³. A second monoclonal antibody, B₁A₃, recognizes the heparin-binding domain of N-CAM and also inhibits cell-substratum adhesion⁹. Retinal cell monolayers were incubated with these monoclonal antibodies, washed, and incubated with labelled probe cells. In some cases Fab fragments of the monoclonal antibody were present throughout the assay. The C₁H₃ monoclonal inhibits cell-cell adhesion by approximately 70% (Table 2), which indicates that in this assay system the rate of cell-cell adhesion is to a large extent controlled by N-CAM. Fab fragments of B₁A₃ also inhibit cell-cell adhesion. We have previously shown that the B₁A₃ monoclonal inhibits cell-substratum adhesion more efficiently when present in the assay medium⁹. Here we find that the inhibition of cell adhesion was greater, approaching that observed with heparin, when B₁A₃ Fab fragments were present in the medium than when the retinal cell monolayers were

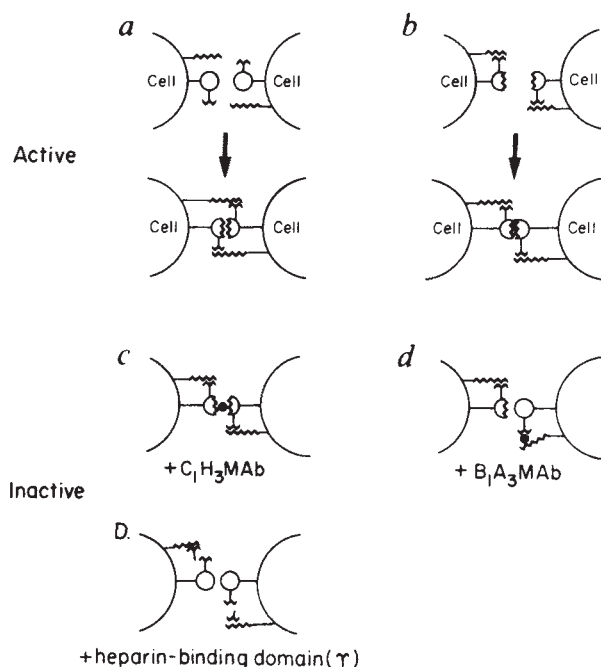


Fig. 2 Schematic diagram depicting possible mechanisms for the interaction between heparan sulphate and N-CAM in neuronal cell-cell adhesion. The model proposes that N-CAM undergoes a conformational change following heparan sulphate binding¹², with homophilic binding between N-CAM molecules resulting in cell-cell adhesion. In *a* and *b*, the model indicates that heparan sulphate-N-CAM binding can be *trans* (N-CAM binds to heparan sulphate on opposing cell) or *cis*, although we favour the former mode of binding. *c-e* show possible mechanisms for the inhibition of cell-cell adhesion. *c*, Inhibition by the C₁H₃ monoclonal, which disrupts homophilic binding; *d*, inhibition by the B₁A₃ monoclonal, which prevents binding of heparan sulphate to N-CAM and thus the conformational change in the protein; *e*, inhibition by heparin-binding domain of N-CAM, which also prevents the conformational change in N-CAM.

washed after exposure to the antibody. As the B₁A₃ monoclonal binds to the heparin-binding domain of N-CAM, these observations directly implicate the binding of heparin to N-CAM in retinal cell-cell adhesion.

We also examined whether the heparin-binding domain isolated from N-CAM could inhibit cell-cell adhesion, as this would provide further support for the role of heparin binding to N-CAM in mediating cell to cell adhesion. The heparin-binding domain of N-CAM has been identified by limited proteolysis of immunopurified N-CAM, followed by chromatography on a heparin-agarose column⁹. The isolated heparin-binding domain is a 25,000-dalton fragment that inhibits cell-substratum adhesion when incubated with retinal cells⁹ and promoted cell-substratum adhesion when covalently coupled to inert surfaces⁹. In the present study ³⁵S-methionine-labelled retinal probe cells were incubated with 50 µg ml⁻¹ of the isolated heparin-binding N-CAM fragment, and then added to mini-vials containing retinal cell monolayers. Cell-cell adhesion was partially inhibited by this polypeptide fragment (Table 2), similar to the inhibition of cell-substratum adhesion previously reported. This implies that the fragment at the concentrations employed⁹ does not fully saturate the cell surface heparan sulphate, which is still available to interact with N-CAM in the adherens¹⁵ or on neighbouring retinal cells.

These data still leave open the possibility that the heparin-binding domain of N-CAM is itself not involved in cell-cell adhesion, but binds to cell surface heparan sulphate that interacts with another heparin-binding component participating in cell adhesion. Because the B₁A₃ monoclonal, which recognizes this polypeptide fragment, inhibits cell-cell adhesion (see

above), it is likely that the heparin-binding domain of N-CAM is an integral component of N-CAM-mediated cell adhesion.

In conclusion, we have demonstrated that cell-cell adhesion in the embryonic chicken neural retina can be inhibited specifically by heparin. A monoclonal antibody that recognizes the heparin-binding domain of N-CAM and a 25,000-dalton heparin-binding polypeptide fragment derived from N-CAM also inhibit cell-cell adhesion. These data suggest that retinal cell adhesion depends upon N-CAM interactions with heparan sulphate, and that these interactions are required for N-CAM homophilic binding. Previous studies in our laboratory have suggested that the binding of heparan sulphate to N-CAM induces a conformational change in the protein¹³, which could modulate homophilic binding between N-CAM molecules. A possible mechanism for the modulation of cell adhesion by heparan sulphate is shown in Fig. 2. Other laboratories have demonstrated that the heparin-binding domains of fibronectin and laminin can promote neurite outgrowth^{16,17}. The multiple roles for cell surface proteoglycans require further detailed exploration, in particular to ascertain if other cell adhesion molecules are also able to interact with heparan sulphate, as this would imply that heparan sulphate has a critical regulatory role in a variety of cell interactions during development.

This work was supported by grants GM-81405 and EY-0566 from the National Institutes of Health.

Received 30 October 1985; accepted 13 February 1986.

1. Rakic, P. & Sidman, R. L. *J. comp. Neurol.* **152**, 103-161 (1973).
2. Rutishauser, U., Grumet, M. & Edelman, G. M. *J. Cell Biol.* **97**, 145-152 (1983).
3. Thiery, J. P., Brackenbury, R., Rutishauser, U. & Edelman, G. M. *J. biol. Chem.* **252**, 6841-6845 (1977).
4. Jorgensen, O. S., Delouves, A., Thiery, J. P. & Edelman, G. M. *FEBS Lett.* **111**, 39-42 (1980).
5. Hirn, M., Ghandour, M. S., Deagostini-Bazin, H. & Goridis, C. *Brain Res.* **265**, 87-100 (1983).
6. Edelman, G. M. *Science* **219**, 450-457 (1983).
7. Rutishauser, U. *Nature* **310**, 549-554 (1984).
8. Rutishauser, U., Hoffman, S. & Edelman, G. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 685-689 (1982).
9. Cole, G. J. & Glaser, L. *J. Cell Biol.* **102**, 403-412 (1986).
10. Cole, G. J. & Glaser, L. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2260-2264 (1984).
11. Cole, G. J. & Glaser, L. *J. biol. Chem.* **259**, 4031-4034 (1984).
12. Cole, G. J. & Glaser, L. *J. Cell Biol.* **99**, 1605-1612 (1984).
13. Cole, G. J., Schubert, D. & Glaser, L. *J. Cell Biol.* **100**, 1192-1199 (1985).
14. Gottlieb, D. I., Rock, K. & Glaser, L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 410-414 (1976).
15. Schubert, D. & LaCorbiere, M. *J. Cell Biol.* **100**, 56-63 (1985).
16. Edgar, D., Timpl, R. & Thoenen, H. *EMBO J.* **3**, 1463-1468 (1984).
17. Rogers, S. L., McCarthy, J. B., Palm, S. L., Furcht, L. T. & Letourneau, P. C. *J. Neurosci.* **5**, 369-378 (1985).

Expression of N-cadherin adhesion molecules associated with early morphogenetic events in chick development

Kohei Hatta & Masatoshi Takeichi*

Department of Biophysics, Faculty of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606, Japan

Selective adhesive properties of cells are thought to have a key role in animal morphogenesis¹, but the molecular bases underlying these properties remain to be determined. Our studies have demonstrated that cell-type-specific adhesiveness resides in a class of cell-cell adhesion molecules, termed cadherins, which were defined as the molecular components of the Ca²⁺-dependent cell adhesion system (CADS)^{2,3}. For example, a cadherin molecule identified in mouse teratocarcinoma cells, termed E-cadherin (this molecule seems to be identical to uvomorulin⁴ or cell-CAM 120/80 (ref. 5) and equivalent to chicken L-CAM⁶), was detected only in epithelial cells of various organs^{2,3}; it did not cross-react with cadherins on other cell types^{7,8}. We recently described a novel type of cadherin, N-cadherin, which is found in mouse cells and whose tissue distribu-

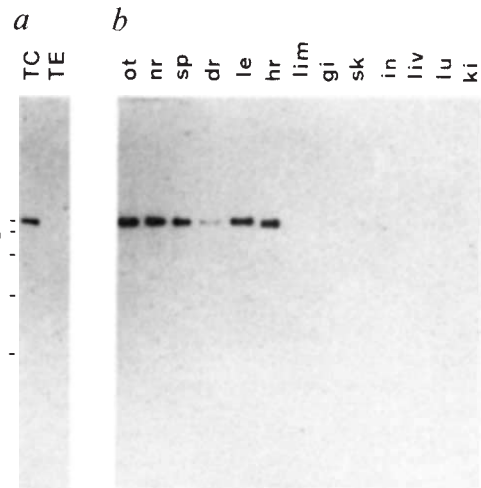


Fig. 1 Immunoblot analysis of antigens recognized by antibody NCD-2. *a*, Neural retina treated either with a 0.01% trypsin solution containing 10 mM CaCl₂ (ref. 13) (TC) or with the same trypsin solution containing 1 mM EGTA instead of CaCl₂ (TE), for 20 min at 37 °C. *b*, Various non-trypsinized tissues: ot, optic tectum; nr, neural retina; sp, spinal cord; dr, dorsal root ganglion; le, lens; hr, heart; li, limb without skin; gi, gizzard; sk, abdominal skin; in, intestine; liv, liver; lu, lung; ki, kidney. *M_r* markers: G, β-galactosidase (116K); P, phosphorylase *b* (94K); A, serum albumin (68K); O, ovalbumin (43K).

Methods. Tissues collected from 6-day-old chick embryos were dissolved in SDS and subjected to SDS-polyacrylamide gel electrophoresis. Proteins were blotted onto nitrocellulose sheets as described elsewhere², then the nitrocellulose sheets were incubated with a culture supernatant of NCD-2 hybridoma, followed by ¹²⁵I-labelled anti-rat antibody (Amersham), then processed for autoradiography². NCD-2 was obtained as follows: Neural retina and brain tissues were collected from two 6-day-old chick (White Leghorn) embryos, treated with TC, then homogenized with Freund's complete adjuvant. The homogenates were injected intraperitoneally (i.p.) into a Wistar rat; after 22 days, the rat was given a second i.p. injection comprising the same tissues (collected from six embryos) treated with TC and homogenized with Freund's incomplete adjuvant. Splenocytes of this animal were fused with P3-X63-Ag-U1 myeloma cells 3 days after the second injection. For screening of hybridomas, we used the following immunoblot analysis. Proteins of neural retina treated with TC or TE from 6-day-old chick embryos were electrophoresed and transferred to nitrocellulose sheets. The sheets were cut into thin strips and each strip was incubated with culture supernatants derived from different hybridoma clones. After detection of antigen bands using an appropriate second antibody, the bands detected in TC-treated samples were compared with those detected in TE-treated samples for each hybridoma supernatant. Hybridoma-producing antibodies which reacted with components present only in TC-treated tissue were saved. One such antibody was NCD-2.

tion is distinct from that of E-cadherin³. In the present study, we have identified a molecular component of N-cadherin in the chicken and determined its distribution in the tissues of early embryos. The results suggest that expression of this adhesion molecule is associated with separation and sealing of cell layers in morphogenesis.

The Ca²⁺-dependent cell adhesion system displays a unique trypsin sensitivity in that it is removed from cell surfaces by treatment with trypsin in the presence of EGTA (TE treatment) but left intact after treatment with trypsin in the presence of Ca²⁺ (TC treatment)⁹⁻¹². We obtained several monoclonal antibodies which react with cell surfaces of chick neural retina dissociated by TC treatment but not with those dissociated by TE treatment. Immunoblot analysis showed that all these antibodies recognize a single component with a relative molecular mass (*M_r*) of 127,000 (127K) (Fig. 1*a*). We chose one of these monoclonal antibodies (NCD-2) for further studies. NCD-2

* To whom correspondence should be addressed.

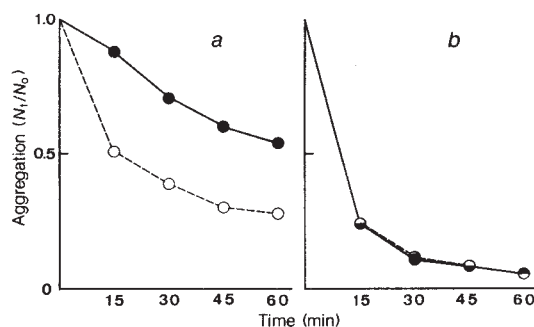


Fig. 2 Effect of NCD-2 on aggregation of neural retina cells. Tissues were collected from 6-day-old chick embryos and dissociated by treatment with TC (a) or LTE¹³ (b). Cells were allowed to aggregate in either the presence (●) or absence (○) of purified NCD-2 antibody (25 µg ml⁻¹). The extent of aggregation was measured using a Coulter counter and is represented by an index N_t/N_0 , as defined elsewhere¹³. (See ref. 13 for details of the methods of dissociation and aggregation of neural retina cells.) As a control experiment, we tested the effect of a rabbit antiserum raised against whole chicken neural retina, which did not contain antibodies recognizing the 127K protein. Fab fragments of antibodies purified from this antiserum did not inhibit aggregation of TC-treated neural retina cells, although they bound strongly to the surfaces of the cells. (In these studies we did not use Fab fragments of the NCD-2 antibody as even its intact form inhibited cell aggregation. This antibody seems unable to agglutinate cells, therefore the assay of inhibition of cell aggregation by the antibody was not hindered by agglutination. A similar property was observed for the ECCD-1 antibody².)

inhibited the Ca²⁺-dependent aggregation of neural retina cells dissociated by TC treatment, but did not inhibit the Ca²⁺-independent aggregation of neural retina cells dissociated by treatment with a low concentration of trypsin in the presence of EGTA (LTE treatment) (Fig. 2). (The LTE treatment is known to inactivate the CADS but to leave intact the Ca²⁺-independent adhesion system¹³.) These results suggested that NCD-2 specifically recognizes the CADS, and that the 127K protein is a component of this adhesion system.

To provide a rough evaluation of the tissue distribution of the 127K protein, SDS-lysates of various tissues from 6-day-old chick embryos were subjected to immunoblot analysis. Figure 1b shows that all nervous tissues tested, for example, optic tectum, spinal cord, dorsal root ganglia as well as neural retina, contained large amounts of 127K protein. Heart and lens were also strongly positive in reaction with NCD-2. On the other hand, limb, gizzard, skin, intestine, liver, lung and kidney were negative or only weakly positive. Immunohistochemical studies showed that essentially all cells in the nervous tissues and lens as well as all myocardiocytes in the heart were stained by NCD-2. In the weakly positive tissues, only particular groups of cells, such as mesothelium and ganglion clusters, were stained. These results will be described in detail elsewhere (in preparation). Limbs in embryos at this stage contained only a very small amount of the 127K protein; however, at later stages, when myotubes had differentiated, these multinucleated cells became positive. Thus, the tissue distribution of NCD-2 targets in chicken embryos is similar to that of N-cadherin defined in mouse embryos³, suggesting that the 127K protein is the chicken equivalent of mouse N-cadherin. We therefore call this 127K protein chicken N-cadherin.

To investigate the possible morphogenetic role of N-cadherin, we studied the pattern of expression of this adhesion molecule in developing chicken embryos by immunocytochemistry. N-cadherin was not expressed in blastoderm at the blastula stage; it was expressed for the first time at the primitive-streak stage. While the epiblast cells were not stained by NCD-2, cells migrating into the lower layer through the primitive streak did become stained (Fig. 3a).

At the head-folding stage, ectoderm cells remained unstained

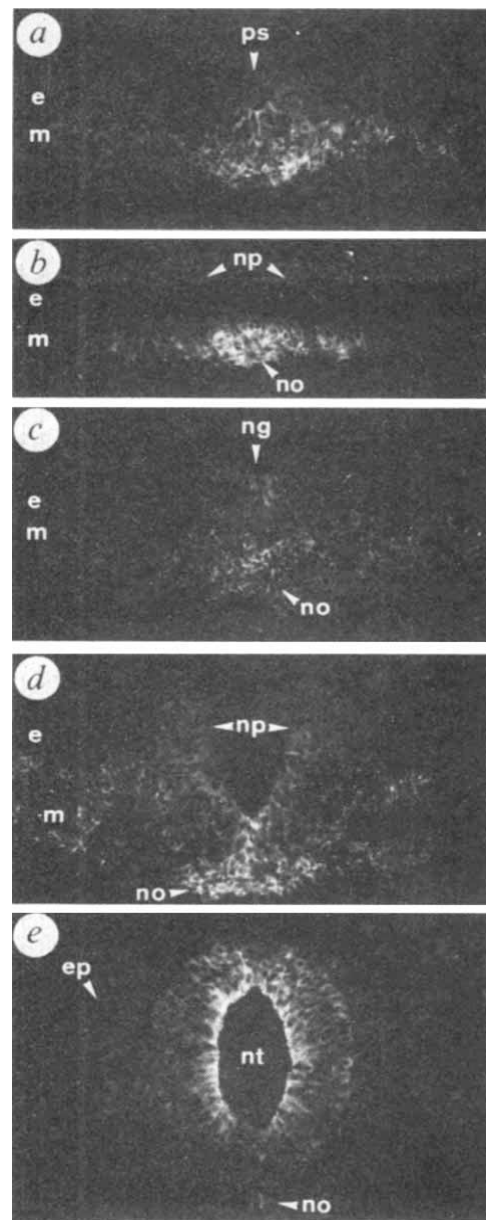
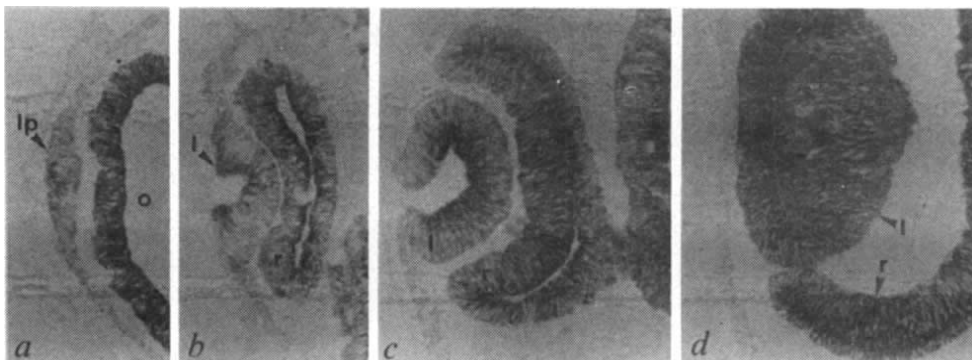


Fig. 3 Immunocytochemical detection of N-cadherin in early embryos at various stages. a, The definitive streak stage. b, The head-process stage; sectioned at the neural plate level. c, 8-somite stage; sectioned at the level between the 8th somite and Hensen's node. d, 11-somite stage; sectioned at a level similar to that in c. e, 8-somite stage; sectioned at the head region. All specimens were transverse sections. e, Epiblast or ectoderm; m, mesoderm and endoderm; ep, epiblast; ps, primitive streak; np, neural plate; ng, neural groove; nt, neural tube; no, notochord. $\times 135$.

Methods. For immunohistochemistry, embryos were fixed in 4% paraformaldehyde in Hanks' solution for 1 h at 4 °C. After incubation in 12–18% sucrose in Hanks' solution for several hours, the samples were layered between abdominal muscle of adult mouse and frozen in isopentane maintained in liquid nitrogen as described by Thierly *et al.*¹⁴. Cryostat sections (10 µm thick) were mounted on slides coated with gelatin and dried in air. The samples were incubated successively in the following solutions, with washing at each interval: (1) in 1% bovine serum albumin in HEPES-buffered Hanks' solution (HHS) for 15 min, (2) in culture supernatant of hybridoma for 30 min, (3) in biotinylated anti-rat antibody (Amersham) diluted 1:100 with HHS for 30 min, and (4) in fluorescein-streptavidin (Amersham) diluted 1:400 with HHS for 15 min. After mounting with 90% glycerol–10% HHS containing 0.1% *p*-phenylenediamine¹⁴, photographs were taken using a Zeiss 18FL microscope. Specificity of antibody binding was checked by comparing the samples with a control that had been treated with ECCD-1 antibody² instead of NCD-2. The ECCD-1 antibody also served as a control in the other experiments described here.

Fig. 4 Immunocytological detection of N-cadherin in the developing eye. *a*, Lens placode; *b* and *c*, invaginating lens vesicle; *d*, lens whose formation was complete. lp, Lens placode; l, lens vesicle or lens; o, optic vesicle; r, retina. Sections were processed as described in Fig. 2 legend, except that fluorescein-streptavidin was replaced by streptavidin-biotin peroxidase complex (Amersham). The distribution of the antigen was visualized by means of the peroxidase reaction as described elsewhere¹⁵. $\times 135$.



until folding of the neural plate began (Fig. 3*b*). When invagination of the neural plate had begun, cells in the deepest portion of the neural groove became stained (Fig. 3*c*), and subsequently the stain extended from the bottom to the top of the folding plate (Fig. 3*d*). At the stage when the neural plate had closed, forming a tube, and was detached from the overlying ectoderm, all cells in the tube were stained (Fig. 3*e*), however the ectoderm layer remaining on the body surface did not take up stain. Mesodermal cells at this stage differentiate into various tissues such as notochord, somites and lateral plate, which became more intensely stained, although some cells such as endothelial cells of blood vessels were not stained. The endoderm layer was also stained.

In embryos at later stages, we found that expression of N-cadherin is associated with a variety of morphogenetic events. From among these findings, we describe here an observation on lens formation. Details of other observations will be published elsewhere (in preparation). The lens is formed by invagination of the ectoderm. When the lens placode formed, this thickened part of the ectoderm became stained by NCD-2, while other parts of the ectoderm surrounding the placode remained unstained or were only weakly stained (Fig. 4*a*). Staining of the lens primordium became more intense as invagination proceeded, although the overlying ectoderm contiguous to the lens vesicle was also weakly stained at the ventral portion (Fig. 4*b, c*). When lens formation was complete, the lens was heavily stained whereas the overlying ectoderm which differentiated into the corneal epithelium was not stained (Fig. 4*d*). In these studies, we found that, in closing or closed vesicular or tubular structures such as neural tube, somite and lens vesicle, the NCD-2 staining was always more intense in the inner portion of the cell layers (see, for example, Figs 3*e, 4*).

Thus, expression of N-cadherin in chicken embryo coincides with various morphogenetic events—gastrulation, neurulation and lens formation. In these three cases, expression of N-cadherin was initiated in cells undergoing separation from other cell layers (that is, in mesoderm and endoderm cells segregating from the epiblast cell layer, and in the neural tube and the lens vesicle as these tissues separated from the overlying ectoderm). Note that the patterns of N-cadherin expression appear to be complementary to those of L-CAM^{6,14}, which is probably the chicken equivalent of mouse E-cadherin¹⁵. According to Thiery *et al.*¹⁴, L-CAM is expressed in the epiblast (upper layer) of blastoderm but not in the mesodermal and endodermal cells migrating into the lower layer of the blastoderm during gastrulation. While L-CAM is permanently expressed in the body-surface ectoderm, it disappears from the neural tube and lens vesicle during their invagination from the overlying ectoderm¹⁴.

Thus, invaginating cells involved in organogenesis of certain tissues begin to express N-cadherin while terminating L-CAM expression. As we reported previously⁸, cells expressing E-cadherin (=L-CAM) do not cross-adhere with cells expressing N-cadherin in a certain cell combination. It seems, therefore, that switches in expression of cadherin from E- to N-type in

the invaginating cells may be an essential step for their segregation from cell layers continuously expressing E-cadherin.

We thank Professor T. S. Okada for his encouragement during this project. This work was supported by research grants from the Japanese Ministry of Education, Science and Culture.

Received 28 June; accepted 21 October 1985.

1. Townes, P. L. & Holtfreter, J. *J. exp. Zool.* **128**, 53–120 (1955).
2. Yoshida-Noro, C., Suzuki, N. & Takeichi, M. *Dev. Biol.* **101**, 19–27 (1984).
3. Hatta, K., Okada, T. S. & Takeichi, M. *Proc. natn. Acad. Sci. U.S.A.* **82**, 2789–2793 (1985).
4. Peyrieras, N., Hyafil, F., Louvard, D., Pleogh, H. L. & Jacob, F. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6274–6277 (1983).
5. Damsky, C. H., Richa, C., Solter, D., Knudsen, K. & Buck, C. A. *Cell* **34**, 455–466 (1983).
6. Edelman, G. M., Gallin, W. J., Delouee, A., Cunningham, B. A. & Thiery, J. P. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4384–4388 (1983).
7. Takeichi, M., Atsumi, T., Yoshida, C., Uno, K. & Okada, T. S. *Dev. Biol.* **87**, 340–350 (1981).
8. Takeichi, M., Hatta, K. & Nagafuchi, A. *UCLA Symp. molec. cell. Biol. new Ser.* **31**, 223–233 (1985).
9. Takeichi, M. *J. Cell Biol.* **75**, 464–474 (1977).
10. Grunwald, G. B., Geller, R. L. & Lilien, J. *J. Cell Biol.* **85**, 766–776 (1980).
11. Brackenbury, R., Rutishauser, U. & Edelman, G. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 387–391 (1981).
12. Magnani, J. L., Thomas, W. A. & Steinberg, M. S. *Dev. Biol.* **81**, 96–105 (1981).
13. Urushihara, H., Ozaki, H. S. & Takeichi, M. *Dev. Biol.* **70**, 206–216 (1979).
14. Thiery, J. P., Delouee, A., Gallin, W. J., Cunningham, B. A. & Edelman, G. M. *Dev. Biol.* **102**, 61–78 (1984).
15. Ogou, S., Yoshida-Noro, C. & Takeichi, M. *J. Cell Biol.* **97**, 944–948 (1983).

Induction of tolerance by monoclonal antibody therapy

R. J. Benjamin & H. Waldmann

Department of Pathology, University of Cambridge,
Tennis Court Road, Cambridge CB2 1QP, UK

A major goal in immunology has been to find a means of selectively abolishing an individual's potential to mount an immune response to certain antigens, while preserving responsiveness to others. The facility to induce such specific immunological unresponsiveness in an adult would have major implications for tissue-grafting, the control of allergy and for treatment of autoimmune disease. Classical work has shown that immunosuppressive regimes, such as irradiation, anti-lymphocyte globulin or thoracic duct drainage, may facilitate tolerance induction^{1,2}. We describe here a technique by which the immune system of mice can be manipulated to be tolerant to certain protein antigens by administering these during a brief pulse of treatment with a monoclonal antibody directed to the L3T4 molecule on helper T lymphocytes. This technique has the potential to form the basis of a novel generalized means of tolerance induction.

The treatment of mice with the rat monoclonal antibodies YTS 191.1 (ref. 3) and GK 1.5 (ref. 4), directed to the L3T4 molecule on T-helper cells⁵, leads to rapid depletion of this subset from the blood and lymphoid tissues^{3,6}, with subsequent immunosuppression of a range of immune functions^{3,6,7}. Given that L3T4-depleted animals fail to respond on immunization

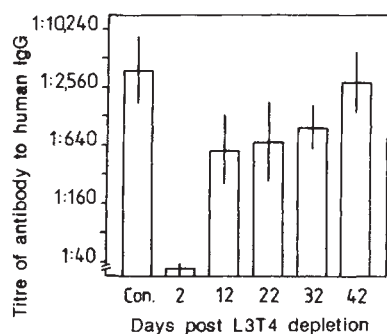


Fig. 1 Return of immunocompetence after YTS 191.1 treatment in euthymic mice.

Methods. Male CBA/Ca mice aged 8–12 weeks were depleted of L3T4-positive T cells by daily injections on days 0 (intravenous (i.v.)), 1 and 2 (intraperitoneal (i.p.)) of YTS 191.1 as 0.2 ml of ascitic fluid from (DA $\times$ LOU) F_1 rat enriched for immunoglobulin by precipitation with 50% saturated ammonium sulphate, and equivalent to ~0.4 mg of active monoclonal antibody per dose. Controls received saline by the same protocol. Groups of four mice were randomly selected on various days following treatment and immunized with 0.5 mg heat-aggregated HGG i.v., purified from group O rhesus-positive serum by ammonium sulphate precipitation followed by elution through an ion-exchange chromatographic column (DE 52, Whatman) in 0.01 M sodium phosphate buffer pH 8.0, dialysed into phosphate-buffered saline (PBS) at 10 mg ml $^{-1}$, and then aggregated by heating to 63 °C for 25 min followed by overnight incubation on ice 1 . Mice were bled from their tail veins 11 days after immunization and the sera stored at -20 °C. Serum anti-HGG antibody titres were measured by an enzyme-linked immunosorbent assay (ELISA) as follows. Polyvinyl micro-titration trays were coated with purified HGG by incubation of 50 μ l per well of 20 μ g ml $^{-1}$ HGG in PBS for 60 min at 37 °C, washed three times in PBS/0.05% (v/v) Tween 20 (Sigma), and then blocked overnight with 1% (w/v) bovine serum albumin (BSA) in PBS/0.02% (w/v) sodium azide. The trays were then washed and doubling dilutions of test sera in 0.1% BSA/PBS were added to each tray at 50 μ l per well and incubated for 60 min at 20 °C. A positive control of peroxidase-linked rabbit anti-human immunoglobulin (Dako) was titrated on each tray. The test samples were followed sequentially, with intervening washes, by species-specific biotinylated sheep anti-mouse immunoglobulin (Amersham) and then biotinylated streptavidin horseradish peroxidase complex (Amersham) both at 1:1,000 dilution in PBS/0.1% BSA and incubated at 50 μ l per well for 35 min at 20 °C. Trays were then washed and developed for 5 min with *o*-phenylenediamine (100 μ l per well) and the reaction stopped with 50 μ l per well of 0.5 M H $_2$ SO $_4$. Absorbance was read at 490 nm, the results plotted graphically and the titres read relative to the positive control. Data are geometric mean $\pm$ s.d. of the antibody titres from four mice.

with protein antigens 6 , we asked whether their immune system maintained any record of such an abortive encounter. This question could be answered by exposing mice to primary antigen challenge during a brief period of anti-L3T4 therapy, and then offering a second challenge dose of the antigen when immunocompetence had been fully re-established. Three responses were possible. First, the animals might appear naive, simply producing a primary-type response. Second, they might have developed memory for a secondary response without exhibiting a primary response. Finally, they might have registered their first encounter with antigen in a negative way and become tolerant.

The experimental design required that we assess the record of antigen encounter in anti-L3T4-treated mice only when full immunocompetence had been restored. Figure 1 shows that mice challenged with heat-aggregated human γ -globulin (HGG) 2 days after L3T4 depletion generated no primary antibody response (as expected). However, challenge at day 12 elicited a strong anti-HGG response and at day 42 this response was

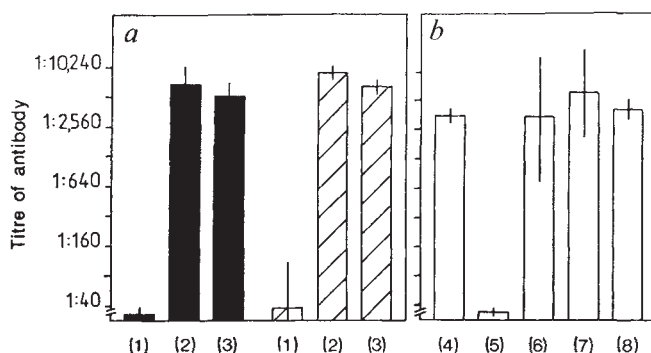


Fig. 2 YTS 191.1 induces unresponsiveness to its own IgG2b epitopes and to heat-aggregated HGG given concomitantly. *a*, Response to rat IgG2b; *b*, response to HGG.

Methods. Adult CBA/Ca male mice in groups of five were pretreated with three injections of YTS 191.1 as in Fig. 1 (groups 1, 4, 5), or as controls received an irrelevant IgG2b monoclonal antibody (YTH 65.3.3, anti-human T200 16) (groups 2, 6) or saline (groups 3, 7, 8) by the same protocol. In *b*, groups 5, 6 and 7 were then injected on days 2 and 3 with 0.5 mg doses of heat-aggregated HGG prepared as in Fig. 1. Following this pretreatment, mice were maintained on antibiotics (oxytetracycline 50 mg l $^{-1}$, Terramycin; Pfizer) and were free of any obvious disease. On day 12 all mice were bled and the effectiveness of anti-L3T4 treatment was confirmed by the absence of an antibody response to HGG in group 5, as compared with strong primary responses in groups 6 and 7 as measured by an ELISA (data not shown). On days 42 and 52, mice were immunized i.p. with 0.5 mg of the heat-aggregated form of: ■, YTH 3.2.6 (anti-CD7 (Campath 2 10) IgG2b); ▨, YTH 53.1.4 (anti-human red blood cell (H.W., unpublished data), IgG2b); or □, HGG. YTH 3.2.6 and YTH 53.1.4 were purified from (DA $\times$ LOU) F_1 rat ascites by ammonium sulphate precipitation, followed by ion-exchange chromatography in 0.01 M sodium phosphate buffer pH 8.0 (DE 52, Whatman) and then heat-aggregated as above. Mice were bled from their tail veins on day 58 and their sera stored at -20 °C. The serum titres of antibody to the respective immunizing Ag (YTH 3.2.6, YTH 53.1.4 or HGG) were determined by ELISA as in Fig. 1. A mixture of mouse monoclonal antibodies against rat immunoglobulin (Norig 7.16.2, Norig 1.1.6 [both anti-rat IgG2b 17] and Mar 18.5 [anti-rat light chain 18]) was used as a reference-positive control for measurement of the anti-rat IgG2b antibody responses. Data are geometric means $\pm$ s.d. of antibody titres from five mice.

equivalent to that in untreated controls. Secondary antigen challenge at day 42 after L3T4 treatment would, therefore, give a reliable measure of the immune system's record of its encounter with antigen given under the 'umbrella' of anti-L3T4.

The first test antigen we considered was the anti-L3T4 monoclonal antibody itself. YTS 191.1 and GK 1.5 (both of the rat IgG2b subclass) do not elicit anti-globulin responses *in vivo* 6,8,9 , whereas other rat IgG2b monoclonals with specificity for lymphocytes, all elicit strong responses to themselves 9 . As YTS 191.1 is non-immunogenic, we asked how mice treated with this antibody recorded their encounter with its IgG2b constant-region determinants? In the experiment shown in Fig. 2*a*, mice were pretreated with YTS 191.1, or an irrelevant monoclonal antibody or saline, and then 42 days later were immunized with either of two different heat-aggregated IgG2b monoclonals, having no binding activity for mouse cells (YTH 3.2.6 10 and YTH 53.1.4). No anti-globulin responses could be detected in anti-L3T4-treated mice (group 1) compared with controls (groups 2, 3). Similarly, administration of HGG under the anti-L3T4 umbrella led to later unresponsiveness to rechallenge with this antigen (Fig. 2*b*, group 5). Mice receiving anti-L3T4 but no initial 'recording' dose of HGG (group 4), responded as well as controls (groups 6–8) to the late HGG challenge. Similar results were obtained with HGG derived from a different donor. Later bleeds did not expose immune responses in tolerant mice.

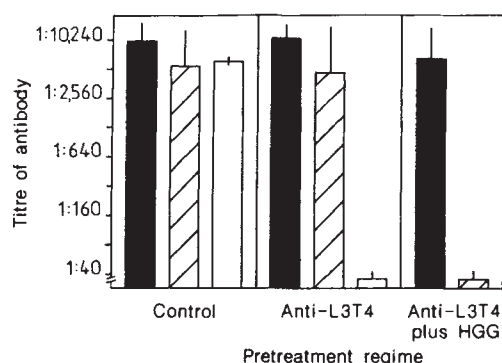


Fig. 3 Specificity of unresponsiveness to rat IgG2b and HGG. Adult male CBA/Ca mice were rendered unresponsive to rat IgG2b by YTS 191.1 (anti-L3T4 antibody) pretreatment as in group 1 in Fig. 2a, or to HGG by pretreatment with YTS 191.1 plus aggregated HGG (anti-L3T4 antibody plus HGG) as in group 5 in Fig. 2b. On days 42 and 52 after induction of unresponsiveness, these mice and age-matched controls were immunized with 0.5 mg i.p. of the heat-aggregated form of HGG (□), CGG (■) or YTH 3.2.6 (▨) (rat IgG2b), and then bled on day 58. Chicken γ -globulin was obtained from Miles Laboratories and heat-aggregated as above. Sera were stored at -20°C and antibody titres determined by ELISA. Data are geometric means $\pm$ s.d. of antibody titres from five mice.

This state of unresponsiveness could be shown to be true tolerance by specificity controls. Mice rendered unresponsive to rat IgG2b by administration of anti-L3T4 remained fully responsive to HGG or chicken γ -globulin (CGG) (Fig. 3). Similarly mice made unresponsive to HGG given under the anti-L3T4 umbrella responded normally to CGG (Fig. 3).

We have shown here that mice injected with antigen under the umbrella of an anti-L3T4 antibody maintain a record of the encounter manifesting as tolerance. As a method of tolerance induction it is unique, as we have been able to induce tolerance in normal adult mice with an immunogenic form of an antigen and there is evidence to suggest a special role for anti-L3T4 in permitting tolerance. Other rat IgG2b monoclonal antibodies that deplete lymphocyte populations (such as anti-Thy 1 or anti-Lyt 1) are also immunosuppressive, yet elicit strong anti-globulin responses to themselves⁹, and are therefore unlikely to permit tolerance to other antigens.

T-helper cell depletion by YTS 191.1 may leave up to 10% of the L3T4⁺ cells in peripheral lymphoid organs³. Why, then, are these remaining cells not primed by antigen to prevent tolerance induction? Perhaps depletion of helper cells is not the only determinant for tolerance induction, and anti-L3T4 monoclonal antibody facilitates tolerance in some other way. Whatever the mechanism, this monoclonal regime has permitted tolerance to rat and human immunoglobulins. However, we have not yet achieved tolerance with heat-aggregated CGG (data not shown). It is likely that this highly immunogenic antigen is able to prime residual T-helper cells and that more aggressive protocols of anti-L3T4 therapy may be necessary to tip the balance of the immunological record towards tolerance.

Our results have direct implications for serotherapy with monoclonal antibodies. First, the anti-globulin response, a major obstacle to any long-term antibody therapy¹¹, could be minimized if it were possible to exploit the tolerogenic properties of anti-L3T4 (CD4¹²) monoclonal antibodies in humans. Second, many recent publications have demonstrated the value of anti-L3T4 monoclonals for the treatment of a number of autoimmune diseases in mice and rats^{8,13-15}. It is now important to establish whether the benefit of anti-L3T4 therapy in autoimmunity is simply due to blanket immunosuppression or to the creation of a tolerogenic milieu in which the immune system relearns 'self' and no longer pursues its auto-aggressive course. If the latter, then anti-L3T4 therapy may prove to be a preferred means of

treatment of clinical autoimmunity, and perhaps of hypersensitivity in general.

The tolerogenic potential of treatment with anti-L3T4 monoclonal antibodies offers some exciting prospects. Not only do we have a simple model for studying basic mechanisms of tolerance, complementing classical systems using deaggregated proteins¹, but perhaps with potentiation of the effects described, anti-L3T4 antibodies may have a future as agents for achieving specific tolerance for therapeutic purposes.

We thank Gilly Martin and Mark Frewin for technical assistance and Drs M. Clark and S. Cobbold for helpful advice and discussion. This work was funded by MRC grants and the Oliver Bird Trust. R.J.B. is funded by the Cambridge Livingstone Trust.

Received 9 January; accepted 24 February 1986.

1. Weigle, W. O. *Adv. Immun.* **16**, 61-121 (1973).
2. Shellam, G. R. *Immunology* **17**, 267-270 (1969).
3. Cobbold, S. P., Jayasuriya, A., Nash, A., Prospero, T. D. & Waldmann, H. *Nature* **312**, 548-551 (1984).
4. Dialynas, D. P. et al. *J. Immun.* **131**, 2445-2451 (1983).
5. Dialynas, D. P. et al. *Immun. Rev.* **47**, 29-56 (1983).
6. Wofsy, D. C., Mayes, J., Woodcock, J. & Seaman, W. E. *J. Immun.* **135**, 1698-1701 (1985).
7. Cobbold, S. P. & Waldmann, H. *Transplantation* (in the press).
8. Wofsy, D. & Seaman, W. E. *J. exp. Med.* **161**, 378-391 (1985).
9. Cobbold, S. P., Martin, G., Lovat, P. E. & Waldmann, H. *Adv. exp. Med. Biol.* **186**, 789-795 (1985).
10. Waldmann, H. et al. *Adv. exp. Med. Biol.* **186**, 869-875 (1985).
11. Ritz, J. & Schlossman, S. F. *Blood* **59**, 1-11 (1982).
12. Reinherz, E. & Schlossman, S. F. *Cell* **19**, 821-826 (1980).
13. Waldor, M. K. et al. *Science* **227**, 415-417 (1985).
14. Ranges, G. E., Sriram, S. & Cooper, S. M. *J. exp. Med.* **162**, 1105-1110 (1985).
15. Brostoff, S. W. & Mason, D. W. *J. Immun.* **133**, 1938-1942 (1985).
16. Bindon, C., Hale, G., Clark, M. & Waldmann, H. *Transplantation* **40**, 538-543 (1985).
17. Hale, G., Clark, M. & Waldmann, H. *J. Immun.* **134**, 3056-3060 (1985).
18. Lanier, L. L., Gutman, G. A., Lewis, D. E., Griswold, S. T. & Warner, N. L. *Hybridoma* **1**, 125-130 (1982).

Abrogation of oral tolerance by contrasuppressor T cells suggests the presence of regulatory T-cell networks in the mucosal immune system

Iwao Suzuki*, Hiroshi Kiyono†, Kyoichi Kitamura*, Douglas R. Green‡ & Jerry R. McGhee*

Departments of * Microbiology and † Oral Biology and Preventive Dentistry, Institute of Dental Research, University of Alabama at Birmingham, University Station, Birmingham, Alabama 35294, USA
‡ Department of Immunology, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

Continuous ingestion of a thymus-dependent (TD) antigen differentially affects two compartments of the immune system. A secretory IgA antibody response is induced in mucosal tissues, concurrent with a state of antigen-specific systemic unresponsiveness to parenteral challenge¹, termed oral tolerance². The precise mechanisms whereby gut antigenic exposure induces oral tolerance are unknown, although T-suppressor cells^{3,4}, anti-idiotypic networks⁵ and immune complex formation⁶ have all been proposed. Here we show that the systemic unresponsiveness of mice made orally tolerant to the TD antigen sheep red blood cells (SRBC) is reversed by the adoptive transfer of Lyt-1⁺, 2⁻, *Vicia villosa* lectin-adherent and I-J⁺ T cells derived from mice which are genetically resistant to the induction of oral tolerance to SRBC. This T-cell subpopulation has the characteristics of contrasuppressor effector T cells (T_{cs}). Small numbers of these T_{cs} cells reverse SRBC-specific tolerance both *in vivo* and *in vitro*. This finding offers new insight into the mechanisms of oral tolerance induction and maintenance, and suggests that a network of T cells are involved in the regulation of host responses to ingested antigens.

Prolonged oral immunization of C3H/HeN mice with SRBC induces systemic unresponsiveness, while identically treated

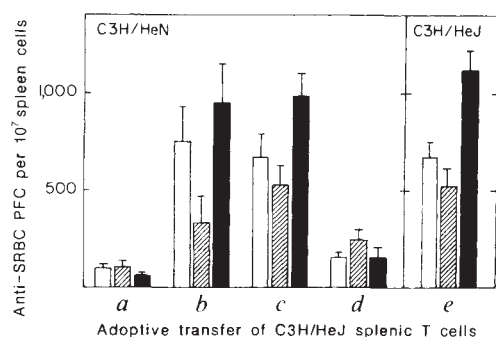
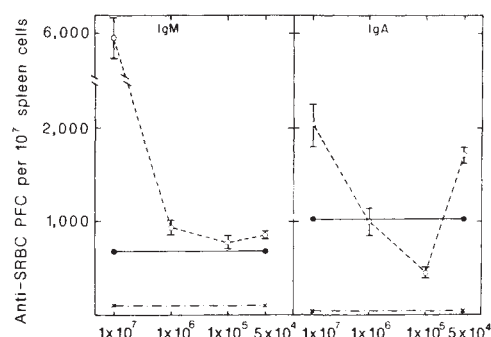


Fig. 1 Reversal of oral tolerance by adoptive transfer of *V. villosa*-adherent T cells. Groups of 22–28 C3H/HeN (a–d) and 18–20 C3H/HeJ (H-2^k) (e) mice were given SRBC daily by GI for 28 days^{3,7}. Seven days later, spleen cells from 8–10 C3H/HeJ mice were enriched for T cells by removal of macrophages by adherence to plastic-coated surfaces and B cells by panning on anti-immunoglobulin-coated plates followed by treatment with anti-immunoglobulin and rabbit C (total T cells). T cells were further fractionated by addition to plates coated with *V. villosa* (L-4600 lot #0127F; E.Y. Laboratories, Inc., San Mateo, California) and non-adherent and adherent T cells were collected^{13,14}. Approximately 7–9% of the T cells adhere to *V. villosa*. Each T-cell fraction (1×10^6 per mouse) was adoptively transferred to groups of 4–6 orally tolerized C3H/HeN mice and immunized i.p. with SRBC. Four days later, splenic anti-SRBC PFC of the IgM (□), IgG (▨) and IgA (■) isotype were determined^{3,7}. a, No T cells; b, total T cells; c, *V. villosa*-adherent T cells; d, *V. villosa*-non-adherent T cells; e, no T cells. Data are taken from four separate experiments.

C3H/HeJ mice exhibit IgM, IgG and IgA anti-SRBC splenic plaque-forming cell (PFC) responses (Fig. 1)³. The inability to induce oral tolerance in C3H/HeJ mice is linked to a defective gene (*Lps^d*) on chromosome 4 which renders lymphoid cells unresponsive to the endotoxin (lipopolysaccharide, LPS)) of Gram-negative bacteria^{7,8}. Analysis of the regulatory T cells in the Peyer's patches and spleens of orally immunized LPS-responsive C3H/HeN (*Lpsⁿ/Lpsⁿ*) mice identified a predominant T-suppressor (T_s) cell activity, while the LPS-nonresponsive C3H/HeJ (*Lps^d/Lps^d*) mice were characterized by a predominant T-helper (T_h) cell activity^{3,7}. This response pattern could result from regulatory T cells in the Peyer's patches of C3H/HeJ mice which render T_h cells resistant to T_s -cell activity, and would, therefore, exhibit the T_{cs} -cell activity described by Gershon, Green and colleagues^{9–13}.

To test this possibility, groups of C3H/HeN and C3H/HeJ mice were orally immunized with SRBC^{3,7} for 28 consecutive days and purified splenic T cells from the C3H/HeJ animals were then adoptively transferred to orally tolerant C3H/HeN mice. Transfer of 1×10^6 C3H/HeJ T cells to the C3H/HeN mice immediately before intraperitoneal (i.p.) challenge abrogated tolerance and allowed the development of IgM, IgG and IgA anti-SRBC PFC responses (Fig. 1). Furthermore, the active T cells could be enriched by adherence to *Vicia villosa* lectin, while the *V. villosa*-non-adherent T cells were not effective at reversing oral tolerance when adoptively transferred to C3H/HeN mice (Fig. 1). Adoptive transfer of *V. villosa* adherent or non-adherent splenic T cells from orally tolerant C3H/HeN mice into the C3H/HeN strain did not reverse oral tolerance. In addition, *V. villosa*-adherent splenic T cells from unimmunized C3H/HeJ or C3H/HeN mice failed to abrogate systemic unresponsiveness when given to orally tolerant C3H/HeN mice. These results suggest that C3H/HeJ mice given SRBC orally develop a subset of splenic T cells which abrogate oral tolerance *in vivo*, and that this T-cell subset shares the property of *V. villosa* binding with the previously described T_{cs} cells^{12,13}.

V. villosa-adherent splenic T cells from C3H/HeJ mice were tested over a wide range of cell number (1×10^7 – 5×10^4), and all cell numbers used reversed oral tolerance upon adoptive transfer to C3H/HeN mice (Fig. 2). Higher numbers of *V. vil-*



Adoptive transfer of C3H/HeJ *V. villosa*-adherent T cells

Fig. 2 Reversal of oral tolerance is achieved with small numbers of *V. villosa*-adherent T cells. Various doses (1×10^7 – 5×10^4 cells) of *V. villosa*-adherent T cells from spleens of 8–10 C3H/HeJ mice given SRBC by GI for 28 days were adoptively transferred to groups of 4–6 orally tolerized C3H/HeN mice. The animals were immunized i.p. with SRBC and splenic IgM and IgA anti-SRBC PFC were determined 4 days later. As controls, groups of 12 C3H/HeJ and C3H/HeN mice were given SRBC by GI for 28 days followed by systemic injection of this antigen 1 week later; (●—●) for C3H/HeJ and (— — —) for C3H/HeN. Data are taken from three separate experiments.

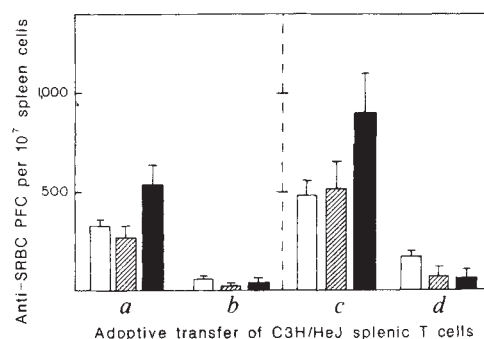


Fig. 3 Contrasuppressor T cells mediate reversal of oral tolerance. Splenic T cells from C3H/HeJ mice given SRBC by GI for 28 days were prepared as described in Fig. 1 legend. T cells were further enriched for T_{cs} by treatment with monoclonal anti-Lyt-2 (clone #53-6-72) followed by addition of anti-rat IgG and C. This treatment removed the T_{cs} T-cell population as determined by immunofluorescence staining with fluorescein isothiocyanate-anti-Lyt-2. These T_{cs} T cells were then treated with anti-I-J^k (Cedarlane) and C. Additional aliquots of T_{cs} T cells were added to *V. villosa* plates and the adherent fractions tested (see Fig. 1 legend). T_{cs} T cells were subsequently treated with anti-Lyt-1 (clone #53-7.313) followed by anti-rat IgG and C. Individual T-cell fractions (5×10^4 cells per mouse) were adoptively transferred to groups of 5–7 orally tolerized C3H/HeN mice and immunized i.p. with SRBC. IgM (□), IgG (▨) and IgA (■) anti-SRBC PFC responses were assessed 4 days later. a, Anti-Lyt-2 + C; b, anti-Lyt-2, anti-I-J^k + C; c, anti-Lyt-2 + C, *V. villosa* adherent; d, anti-Lyt-2 + C, *V. villosa* adherent, anti-Lyt-1 + C. Data are taken from three separate experiments.

losa-adherent T cells gave elevated IgM responses, while 10^5 – 10^6 cells supported responses similar to those seen in C3H/HeJ mice (Fig. 2). Interestingly, when the IgA-isotype response was examined, the transfer of graded numbers of *V. villosa*-adherent T cells resulted in a biphasic response. Both high and low numbers of transferred cells abrogated tolerance, while intermediate numbers were less effective. This may suggest that the functional activity observed is due to two distinct populations of T_{cs} cells, one of which may be IgA-isotype specific.

Enrichment of T_{cs} T cells from the C3H/HeJ splenic T-cell population by anti-Lyt-2 and rabbit complement (C) treatment gave a cell fraction which abrogated oral tolerance when adoptively transferred to C3H/HeN mice (Fig. 3). This cell fraction contains mature T_h cells^{3,7} and effector T_{cs} cells, since further

Table 1 *V. villosa*⁺, Lyt-1⁺, I-J^{k+} T cells abrogate the unresponsiveness of spleen cells from orally tolerized mice *in vitro*

Purified T cells added (cell treatment)		Cell no. added†	Anti-SRBC PFC per culture*		
			IgM	IgG	IgA
None		None	65 ± 11	31 ± 12	37 ± 23
Anti-Lyt-2 + C‡		1 × 10 ⁵	261 ± 36	126 ± 15	263 ± 26
		5 × 10 ⁵	243 ± 35	192 ± 25	332 ± 42
Anti-Lyt-2 and anti-I-J ^k + C‡		1 × 10 ⁵	105 ± 14	33 ± 11	71 ± 9
		5 × 10 ⁵	40 ± 9	52 ± 7	75 ± 4
Anti-Lyt-2 + C	<i>V. villosa</i> adherent§	1 × 10 ⁵	257 ± 24	140 ± 11	261 ± 37
		5 × 10 ⁵	172 ± 22	184 ± 18	279 ± 12
	<i>V. villosa</i> non-adherent§	1 × 10 ⁵	92 ± 6	100 ± 8	62 ± 6
		5 × 10 ⁵	70 ± 8	66 ± 4	33 ± 7
Anti-Lyt-2 and anti-Lyt-1 + C	<i>V. villosa</i> adherent§	1 × 10 ⁵	94 ± 15	46 ± 8	47 ± 7
		5 × 10 ⁵	69 ± 11	34 ± 8	9 ± 20

Spleen cells (5×10^6) from seven to nine C3H/HeN mice orally tolerized with SRBC were cultured in minimal essential medium containing L-glutamine, gentamicin, penicillin, streptomycin, sodium bicarbonate, sodium pyruvate, non-essential amino acids and 10% fetal calf serum and immunized with SRBC (2.5×10^6 per culture) in the presence or absence of added T cells (either 5×10^5 or 1×10^5 per culture) (see below) and incubated for 5 days^{3,7}.

* Values are the mean IgM, IgG or IgA anti-SRBC PFC per culture per experiment and from three separate experiments. Non-immunized, control cultures gave less than 4 anti-SRBC PFC per culture.

† Aliquots of T cells in various treatment groups were titrated over a range of 1×10^4 – 1×10^6 cells per culture. Representative doses of 1×10^5 – 5×10^5 are included. T cells from normal C3H/HeJ spleen were without effect when added to tolerant C3H/HeN splenic cultures.

‡ Splenic T cells from 10 C3H/HeJ mice given SRBC by gastric intubation (GI) daily for 28 days were prepared as described in Fig. 1 legend. T cells were further enriched into Lyt-1⁺ fractions by treatment with anti-Lyt-2 and C (Fig. 3 legend). This treatment removed the Lyt-2⁺ T-cell population as determined by immunofluorescence. Aliquots of Lyt-1⁺ T cells were treated with anti-I-J^k and C.

§ Lyt-1⁺-enriched T cells from C3H/HeJ spleen were separated into adherent and non-adherent fractions on *V. villosa* lectin-precoated plates. Additionally, Lyt-1⁺ *V. villosa*-adherent T cells were treated with anti-Lyt-1 and C (see Fig. 3 legend).

fractionation of Lyt-1⁺ T cells by adherence to *V. villosa* resulted in adherent Lyt-1⁺ T cells with tolerance-reversing activity, while non-adherent enriched Lyt-1⁺ T_h cells were without effect (Fig. 3 and data not shown). In addition, treatment of Lyt-1⁺, *V. villosa*-adherent T cells with anti-Lyt-1 and C abrogated reversal of oral tolerance. This suggests that the active T-cell fraction is Lyt-1⁺, 2⁻ and *V. villosa* adherent, all properties of effector T_{cs} cells⁹⁻¹³.

Previous work has shown that I-J determinants occur on T cells with helper amplifier and contrasuppressor activity⁹⁻¹³ and on T-cell-derived factors responsible for contrasuppression^{9,14}. Although the gene(s) that code for I-J are not known, strong evidence from studies with alloanti-I-J and monoclonal anti-I-J antibodies clearly associate this molecule with T_{cs} cells. When splenic Lyt-1⁺ T-cell-enriched fractions from C3H/HeJ mice were treated with anti-I-J^k and C, T_{cs}-cell activity was completely lost (Fig. 3). We conclude that reversal of oral tolerance in C3H/HeN mice is mediated by Lyt-1⁺, 2⁻, *V. villosa*⁺, I-J^{k+} effector T_{cs} cells.

The T_{cs}-cell fractions prepared by the various treatments described above were also effective in restoring tolerant C3H/HeN spleen cells to IgM, IgG and IgA anti-SRBC PFC responses *in vitro*. Lyt-1⁺, *V. villosa*-adherent T cells supported *in vitro* PFC responses of all isotypes, while the *V. villosa*-non-adherent, Lyt-1⁺ T_h-cell-enriched fraction was ineffective (Table 1). Additional treatment of Lyt-1⁺ T cells with anti-I-J^k and C or with anti-Lyt-1 and C eliminated T_{cs}-cell activity. Thus, studies *in vitro* confirmed our results with adoptively transferred T cells, and clearly showed that the active T cell is Lyt-1⁺, *V. villosa*-adherent and I-J^{k+}.

The present results suggest that regulatory T-T-cell interactions determine the outcome of the host response to systemically administered TD antigen. Mice rendered orally tolerant to SRBC exhibit T_s cells, which override T_h-B-cell interactions and result in unresponsiveness^{3,7}. The finding that active T_{cs} cells from non-tolerant C3H/HeJ mice convert tolerant C3H/HeN mice to responsiveness clearly indicates that oral tolerance to SRBC

is largely mediated by T_s cells. Implicit in this argument is the presence of T_h cells in tolerant mice, which fail to function in the absence of T_{cs} cells, and T_s-cell-regulated systemic unresponsiveness ensues. However, T_{cs} cells, together with residual T_h cells, overcome T_s-cell-mediated tolerance and produce anamnestic-type responses to systemic antigen. Although we do not understand why LPS-nonresponsive C3H/HeJ mice exhibit enhanced T_{cs}-cell activity to orally administered SRBC, it is tempting to suggest that endogenous gut LPS diminishes T_{cs}-cell induction, perhaps via effects on inducer or transducer precursors of T_{cs} effector cells in Peyer's patches of normal LPS-responsive hosts.

Other studies^{9,12} have suggested that T_{cs} cells act on T_h cells to render them resistant to suppressor cell influences, and the studies reported here are consistent with this. Our past work has shown that residual T_h cells occur in both Peyer's patches and spleen of orally tolerant mice^{3,7}; however, suppressor T-cell activity was dominant. These T_h cells, in the presence of effector T_{cs} cells, may be able to bypass oral tolerance. Alternatively, it remains possible that T_{cs} cells act at the level of T_s cells and inhibit their function. In this mode, the T_{cs} cell would also serve as a suppressor cell whose target is another suppressor cell. This may better explain why small numbers of T_{cs} cells can abrogate oral tolerance *in vivo*. In either situation orally induced T_h cells would function to support anamnestic-type antibody responses in all major isotypes.

We thank Drs J. L. Butler and J. H. Eldridge for critical evaluation of this work and Ms Betty Wells for typing the paper. These studies were supported by USPHS grants AI 19674, AI 18958, DE 04217, New Investigator Research Award AI 21032 (to H.K.) and the Alberta Heritage Foundation (to D.R.G.)

Received 5 November 1985; accepted 28 January 1986.

- Challacombe, S. J. & Tomasi, T. B. Jr *J. exp. Med.* **152**, 1459–1472 (1980).
- Tomasi, T. B. Jr *Transplantation* **29**, 353–356 (1980).
- Kiyono, H., McGhee, J. R., Wannemuehler, M. J. & Michael, S. M. *J. exp. Med.* **155**, 605–610 (1982).
- Richman, L. K., Chiller, J. M., Brown, W. R., Hanson, D. G. & Vaz, N. M. *J. Immun.* **121**, 2429–2434 (1978).

5. Kagnoff, M. F. *Gastroenterology* 79, 54-61 (1980).
6. André, C., Heremans, J. F., Vaerman, J. P. & Cambiaso, C. L. *J. exp. Med.* 142, 1509-1519 (1975).
7. Michalek, S. M., Kiyono, H., Wannemuehler, M. J., Mosteller, L. M. & McGhee, J. R. *J. Immunol.* 128, 1992-1998 (1982).
8. Watson, J., Kelly, K., Largen, M. & Taylor, B. *Ann. J. Immunol.* 120, 422-424 (1978).
9. Green, D. R., Flood, P. M. & Gershon, R. K. *A. Rev. Immunol.* 1, 439-463 (1983).
10. Gershon, R. K. *et al. J. exp. Med.* 153, 1533-1546 (1981).
11. Green, D. R., Gold, J., Martin, S. St., Gershon, R. K. *Proc. natn. Acad. Sci. U.S.A.* 79, 889-892 (1982).
12. Green, D. R. *et al. Eur. J. Immunol.* 11, 973-980 (1981).
13. Iverson, M., Ptak, W., Green, D. R. & Gershon, R. K. *J. exp. Med.* 158, 982-987 (1983).
14. Yamauchi, K., Green, D. R., Eardley, D. D., Murphy, D. B. & Gershon, R. K. *J. exp. Med.* 153, 1547-1561 (1981).

Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor

R. J. Gryglewski, R. M. J. Palmer & S. Moncada*

Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, UK

Endothelium-derived vascular relaxing factor (EDRF)¹ is a humoral agent that is released by vascular endothelium and mediates vasodilator responses induced by various substances including acetylcholine and bradykinin². EDRF is very unstable, with a half-life of between 6 (refs 3, 4) and 50 (ref. 5) s, and is clearly distinguishable from prostacyclin⁶. The chemical structure of EDRF is unknown but it has been suggested that it is either a hydroperoxy- or free radical-derivative of arachidonic acid or an unstable aldehyde, ketone or lactone³. We have examined the role of superoxide anion (O_2^-) in the inactivation of EDRF released from vascular endothelial cells cultured on microcarrier beads and bioassayed using a cascade of superfused aortic smooth muscle strips⁷. With this system, we have now demonstrated that EDRF is protected from breakdown by superoxide dismutase (SOD) and Cu^{2+} , but not by catalase, and is inactivated by Fe^{2+} . These findings indicate that O_2^- contributes significantly to the instability of EDRF.

We have recently described a method for the bioassay of EDRF which allows differentiation between the effects of substances on the release, action or stability of EDRF⁷. Briefly, 7-14-day-old cultures of porcine aortic endothelial cells on microcarrier beads are packed into a chromatographic column ($2-6 \times 10^7$ cells per column) and perfused with Krebs' solution (5 ml min^{-1} at 37°C) gassed with 5% $CO_2/95\%$ O_2 containing indomethacin ($5 \mu\text{M}$) to inhibit the biosynthesis of prostanoids. The column effluent is used to superfuse in cascade two to four spirally cut strips of rabbit thoracic aorta which are denuded of endothelium and contracted with the stable 11,9-epoxy-methano analogue of prostaglandin H_2 , U46619 ($30-60 \text{ nM}$), or with phenylephrine hydrochloride ($50-200 \text{ nM}$). The uppermost vascular strip on the bioassay cascade is separated from the cells in the column by a delay of 1 s and the subsequent tissues are separated from each other by delays of 3 s. Nitroglycerin ($20-200 \text{ nM}$) relaxes all tissues in the cascade when administered over the tissues (OT). This compound is used to standardize the sensitivity of the assay tissues because vascular relaxation induced by nitroglycerin and EDRF, but not by prostacyclin or β -adrenergic agonists, is reported to be mediated via activation of smooth muscle guanylate cyclase (refs 9, 10).

Bradykinin ($10-100 \text{ nM}$) OT did not affect the tone of the rabbit thoracic aorta preparations, but when infused through the column (TC), it induced the release of EDRF, which in turn relaxed the aorta preparations and disappeared rapidly during passage down the cascade (half-life $<7 \text{ s}$, Fig. 1). As authentic EDRF is not available for the construction of standard dose-

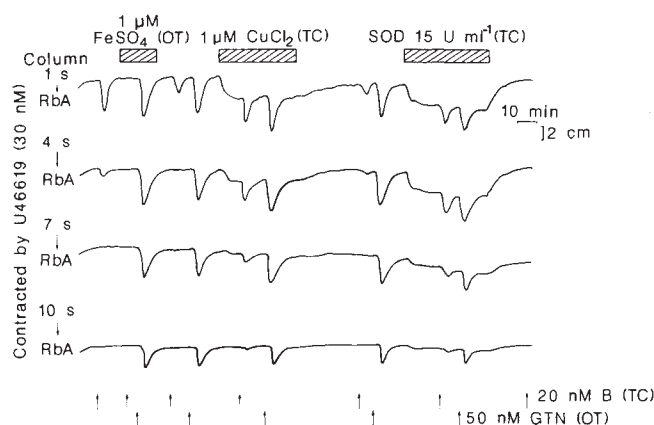


Fig. 1 Effect of Fe^{2+} , Cu^{2+} and SOD on the relaxation of rabbit aortas (RbA) by EDRF. A column packed with a 7-day-old culture of endothelial cells (4.2×10^7 cells) was perfused with Krebs' buffer (5 ml min^{-1}). The effluent was used to superfuse a cascade of four rabbit aortas. These preparations, denuded of endothelium and contracted submaximally by U46619 (30 nM), were separated from the column by delays of 1, 4, 7 and 10 s, respectively. The sensitivity of the rabbit aortas was standardized by administration of nitroglycerin (glyceryl trinitrate, GTN) over the tissues (OT). EDRF was released by 1-min infusions of bradykinin (B, 20 nM) through the column (TC). Infusion of Fe^{2+} ($FeSO_4$, $1 \mu\text{M}$, OT) abolished the vasodilator effect of EDRF without affecting the response to nitroglycerin, indicating the destruction of EDRF by Fe^{2+} . Cu^{2+} ($CuCl_2$, $1 \mu\text{M}$, TC) caused a relaxation of aorta preparations which diminished down the cascade, revealing the spontaneous release of EDRF. In the presence of Cu^{2+} , the EDRF released by bradykinin relaxed all the tissues in the cascade. Therefore, Cu^{2+} increased the survival of EDRF. More than 15 min after the end of the Cu^{2+} infusions, the uppermost aorta had still not fully recovered from the relaxation. SOD (15 U ml^{-1}) infused TC produced a similar effect on the basal and bradykinin-induced release of EDRF which, in contrast to that induced by Cu^{2+} , disappeared within 5 min of terminating the SOD infusion.

response curves, the magnitude of the EDRF-induced relaxations of the vascular strips was expressed as a percentage of that caused by a standard dose of nitroglycerin (50 nM , OT). The relaxation of the first bioassay tissue induced by bradykinin (20 nM , TC)-stimulated EDRF was $111.7 \pm 7.0\%$ (mean $\pm$ s.e.m., $n = 22$) of that of the nitroglycerin standard. These bradykinin-induced vascular relaxations were inhibited 47.3 and 76.5% by haemoglobin ($10 \mu\text{M}$, OT; $n = 2$) and $90.2 \pm 2.0\%$ by methylene blue ($50 \mu\text{M}$, OT; $n = 3$), confirming that the substance released was EDRF¹⁰.

Infusions of SOD ($5-30 \text{ U ml}^{-1}$, TC) caused relaxation of the tissues (Figs 1, 3), the magnitude of the relaxation being greatest in the first assay tissue ($73.0 \pm 6.6\%$ of the nitroglycerin standard, $n = 15$) and decreasing progressively the farther down the cascade the detector tissue was situated. This relaxation was significantly ($P < 0.05$) attenuated when the infusion of SOD was changed from TC to OT ($48.2 \pm 7.5\%$ of the nitroglycerin standard, $n = 6$) and was not observed when the assay tissues were superfused with Krebs' solution instead of with effluent from the column. Furthermore, the relaxations caused by either SOD or bradykinin-induced EDRF were inhibited to a similar extent by methylene blue ($50 \mu\text{M}$, OT) and haemoglobin ($10 \mu\text{M}$, OT). These data suggest that there is a basal release of EDRF from the column which becomes evident when EDRF is stabilized by SOD, so that the release of EDRF induced by bradykinin occurs against a background of continuous release.

The stability of EDRF released by bradykinin was markedly increased by infusions of SOD ($5-30 \text{ U ml}^{-1}$, TC), as shown by the relaxation of the tissues farther down the cascade (Fig. 1). Thus, the relaxations of the first three tissues were increased by 1.4 ± 0.07 ($n = 11$), 4.05 ± 1.00 ($n = 11$) and 9.3 ± 1.3 ($n = 9$) times, respectively. Enhancement of the response of the fourth tissue

* To whom correspondence should be addressed.

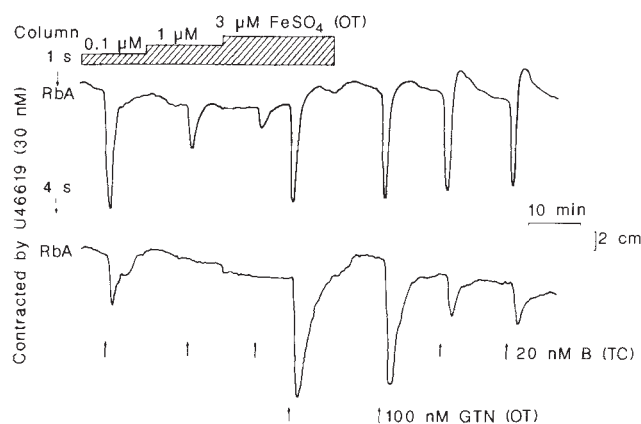


Fig. 2 Effluent of a column containing endothelial cells (9-day-old culture, 2.6×10^7 cells) was used to superfuse two rabbit aorta strips separated by a 3-s delay (as described for Fig. 1). Infusion of increasing concentrations of FeSO_4 (0.1–3 μM , OT) inhibited the effect of EDRF released by bradykinin (20 nM, TC) without affecting the relaxation induced by nitroglycerin (100 nM, OT). This effect disappeared within 10 min of terminating the infusion of FeSO_4 .

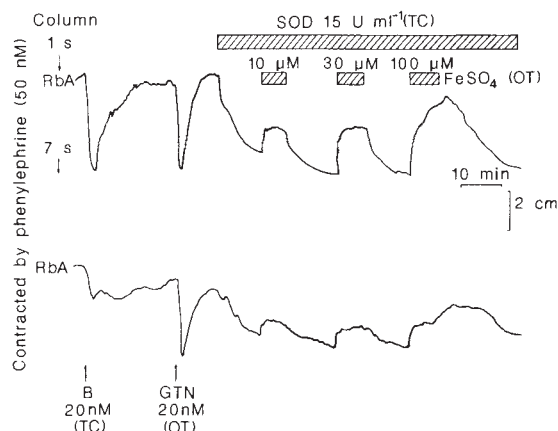


Fig. 3 Effluent of a column containing endothelial cells (13-day-old culture, 5.4×10^7 cells) was used to superfuse two strips of rabbit aorta separated by a 6-s delay (as described in Fig. 1 legend). The tissues were relaxed by bradykinin (20 nM, TC)-induced EDRF and by nitroglycerin (20 nM, OT). Infusion of SOD (15 U ml^{-1} , TC) relaxed the assay tissues. This relaxation could be reversed by infusion of increasing concentrations of FeSO_4 (10–100 μM , OT).

could not be quantified, as EDRF released by bradykinin in the absence of SOD did not reach this tissue. Bradykinin-induced EDRF was also stabilized when the SOD infusion was changed from TC to OT, although in these conditions SOD was less effective (the responses of the first three tissues were enhanced by 1.40 ± 0.16 ($n=5$), 2.70 ± 0.98 ($n=4$) and 3.74 ± 0.97 ($n=3$) times, respectively). The finding that SOD increases EDRF-induced relaxations when given over the tissue indicates that SOD increases the stability of EDRF; whether it also affects EDRF synthesis or release is unclear. The effect of SOD requires its enzymatic activity, as boiled (1 min) enzyme did not enhance EDRF responses.

Catalase (30 U ml^{-1} OT or TC) did not affect the stability of EDRF when given either alone ($n=4$) or in combination with SOD ($n=3$), indicating that H_2O_2 or H_2O_2 -derived radicals do not contribute to EDRF breakdown. Therefore, it appears that O_2^- is a potent inactivator of EDRF and that it is either present or produced in sufficient quantities in our system to inactivate EDRF rapidly.

The idea that the short half-life of EDRF may be a function (at least in part) of the concomitant release of EDRF and O_2^- by vascular endothelial cells merits further study. It may explain the reported differences in the half-life of EDRF^{3,5}, as these may in turn be affected by the ratio of EDRF to O_2^- in a particular experimental system.

EDRF could also be stabilized by Cu^{2+} (CuCl_2 , 1–3 μM , OT), although less effectively than by SOD. Like SOD, CuCl_2 was more effective at protecting EDRF released by bradykinin when the infusion was changed from tissue to column; thus, the bradykinin (20 nM, TC)-induced relaxation of the first three tissues was increased by 1.35 ± 0.03 ($n=4$), 1.70 ± 0.65 ($n=4$) and 2.33 ± 0.90 ($n=3$) times and by 1.50 ± 0.22 ($n=4$), 2.40 ± 0.40 ($n=4$) and 4.2 ± 1.44 ($n=4$) times, respectively, by OT and TC CuCl_2 (1 μM). In addition, infusions of Cu^{2+} alone (TC) caused relaxation of the bioassay tissues (in the uppermost bioassay tissue this relaxation was $48.0 \pm 7.3\%$ ($n=6$) of that induced by the nitroglycerin standard) which was smaller the farther the detector tissue was situated from the column, again suggesting a basal release of EDRF in these conditions. ZnSO_4 (10 μM) did not affect the stability of EDRF in two experiments. Mammalian cytosolic SODs are copper/zinc enzymes, of which copper is the active species¹¹. Since low-relative molecular mass complexes of Cu^{2+} and Cu^{2+} itself mimic SOD activity in specific conditions, this effect of Cu^{2+} is not unexpected. These results suggest that Cu^{2+} acts by dismuting O_2^- directly; whether it also

acts by reactivating¹² exhausted endothelial SOD¹³ is not clear. However, the finding that the protection of EDRF continues for up to 15 min after the end of Cu^{2+} (TC) infusion but not after that of SOD (Fig. 1) supports the latter action. It is interesting that Mn^{2+} (10 μM MnCl_2 , TC), the metal ion in SOD from other sources which can also dismute O_2^- (refs 14, 15), was inactive in two experiments.

Our hypothesis that O_2^- destroys EDRF is further supported by the observation that Fe^{2+} is a potent and selective inhibitor of EDRF. Indeed, FeSO_4 (0.3–10 μM , OT) dose-dependently inhibited the EDRF-induced relaxation of the assay tissues without affecting the response to nitroglycerin (Fig. 2). Thus, 1 μM FeSO_4 OT produced a $78.2 \pm 9.2\%$ ($n=5$) inhibition in the response of the uppermost tissue to bradykinin (20 nM, TC)-stimulated EDRF. In addition, Fe^{2+} reversed the relaxation of the tissues induced by the basal release of EDRF (observed when SOD was given TC). However, in the presence of SOD (15 U ml^{-1} , TC) 10-fold higher concentrations of Fe^{2+} (10–100 μM , OT) were necessary to antagonize the action of EDRF. For example, the relaxation of the uppermost tissue induced by bradykinin-stimulated EDRF was inhibited $95.5 \pm 0.9\%$ ($n=4$) by 10 μM FeSO_4 OT in the absence of SOD and $44.0 \pm 6.5\%$ ($n=3$) in its presence (15 U ml^{-1} , TC). Since Fe^{2+} can catalyse the formation of O_2^- in oxygenated phosphate buffer¹⁶, these findings suggest that Fe^{2+} antagonizes EDRF by inactivating it via the generation of O_2^- . Indeed, the pronounced reduction in potency of Fe^{2+} in the presence of SOD favours this concept (Fig. 3). Fe^{2+} could also inactivate EDRF either directly or by generation of hydroxyl radicals from H_2O_2 in Haber-Weiss and Fenton reactions¹⁷, although the latter explanation would not be consistent with the lack of effect of catalase on EDRF stability.

The effects of Cu^{2+} and Fe^{2+} on EDRF stability may indicate a regulatory role for trace elements in the control of the vascular system in health and disease. Both prostacyclin and EDRF have a homeostatic function in vascular integrity. The findings that lipid peroxides and superoxide ions either prevent the synthesis¹⁸ or destroy the biological activity of these mediators suggest a central role for activated oxygen species in the pathogenesis of vasospasm, thrombosis and atherosclerosis.

We thank Sir John Vane for helpful discussion and Ms L. Pynegar and Mr N. Foxwell for technical assistance.

Since this paper was submitted, we have become aware of a preliminary communication by Vanhoutte and Rubanyi⁹ suggesting that SOD increases the half-life of EDRF.

Received 24 September 1985; accepted 20 February 1986.

1. Furchgott, R. F. & Zawadzki, J. V. *Nature* **288**, 373-376 (1980).
2. Furchgott, R. F. *A. Rev. Pharmac. Tox.* **24**, 175-197 (1984).
3. Griffith, T. M., Edwards, D. H., Lewis, M. J., Newby, A. C. & Henderson, A. H. *Nature* **308**, 645-647 (1984).
4. Cocks, T. M., Angus, J. A., Campbell, J. H. & Campbell, G. R. *J. cell. Physiol.* **123**, 310-320 (1985).
5. Förstermann, U., Trogisch, G. & Busse, R. *Eur. J. Pharmac.* **106**, 639-643 (1985).
6. Moncada, S., Gryglewski, R. J., Bunting, S. & Vane, J. R. *Nature* **263**, 663-665 (1976).
7. Gryglewski, R. J., Moncada, S. & Palmer, R. M. J. *Br. J. Pharmac.* **87**, 685-694 (1986).
8. Vane, J. R. *Br. J. Pharmac. Chemother.* **23**, 360-373 (1964).
9. Rapaport, R. M., Drazin, M. B. & Murad, F. *Nature* **306**, 174-176 (1983).
10. Martin, W., Villani, G. M., Jothianandan, D. & Furchgott, R. F. *J. Pharmac. exp. Ther.* **232**, 708-716 (1985).
11. Keele, B. B., McCord, J. M. & Fridovich, I. *J. biol. Chem.* **246**, 2875-2880 (1971).
12. Heikkilä, R. E. & Cohen, G. in *Superoxide and Superoxide Dismutases* (eds Michelson, A. M., McCord, J. M. & Fridovich, I.) 367-373 (Academic, London, 1977).
13. Block, E. R., Patel, J. M. & Sheridan, N. P. *J. cell Physiol.* **122**, 240-248 (1985).
14. Epel, B. L. & Neumann, J. *Biochim. biophys. Acta* **325**, 520-529 (1973).
15. Lumsden, J. & Hall, D. C. *Biochem. biophys. Res. Commun.* **64**, 595-602 (1975).
16. Michelson, A. M. in *Superoxide and Superoxide Dismutases* (eds Michelson, A. M., McCord, J. M. & Fridovich, I.) 77-86 (Academic, London, 1977).
17. Freeman, B. A. & Crapo, J. D. *Lab. Invest.* **47**, 412-426 (1982).
18. Moncada, S., Gryglewski, R. J., Bunting, S. & Vane, J. R. *Prostaglandins* **12**, 715-733 (1976).
19. Vanhoutte, P. M. & Rubanyi, G. M. *Clin. Res.* **33**, 523A (1985).

Dispersed human immunoglobulin κ light-chain genes

E. Lötscher, K.-H. Grzeschik*, H. G. Bauer, H.-D. Pohlenz, B. Straubinger & H. G. Zachau

Institut für Physiologische Chemie der Universität München, Goethestrasse 33, 8000 München 2, FRG

* Institut für Humangenetik der Universität Münster, Vesaliusweg 12-14, 4400 Münster, FRG

The gene segments encoding the constant and variable regions of human immunoglobulin light chains of the κ type (C_κ , V_κ) have been localized to chromosome 2 (refs 1, 2). The distance between the C_κ and V_κ genes and the number of germline V_κ genes are unknown. As part of our work on the human V_κ locus (refs 3-6 and reviewed in ref. 7), we have now mapped two solitary V_κ gene and a cluster of three V_κ genes to chromosomes 1, 15 and 22, respectively. The three genes that have been sequenced are non-processed pseudogenes, and the same may be true for the other two genes. This is the first time that V -gene segments have been found outside the C -gene-containing chromosomes. Our finding is relevant to current estimates of the size of the V_κ -gene repertoire. Furthermore, the dispersed gene regions have some unusual characteristics which may help to clarify the mechanism of dispersion.

To elucidate the human V_κ locus, we prepared hybridization probes for the four known V_κ subgroups^{3,8,9} and used them to isolate more than 150 different V_κ -gene-containing cosmids from genomic DNA libraries. Several genomic regions each of about 100 kilobases (kb), altogether about 1,200 kb, comprising 75-80 V_κ genes, could be defined with the help of overlapping cosmids and 'genomic walking' experiments (refs 4-6 and H.-D.P. *et al.* and B.S. *et al.*, manuscripts in preparation). Subclones prepared from the various regions were then hybridized to a panel of digested DNAs from human-rodent cell hybrids. As expected, most of the regions mapped to chromosome 2 but three regions were found on chromosomes 1, 15 and 22, respectively (Figs 1, 2); accordingly, we named them Chr1, Chr15 and Chr22.

Genomic regions Chr1 and Chr15 contain V_κ genes of subgroup I according to comparative hybridization experiments of cosmid digests with probes of different V_κ subgroups. Based on a comparison of their restriction maps with the map of a related gene which has been sequenced (R. Thiebe, unpublished), the V_κ I genes are non-processed genes or pseudogenes. Also, the absence of a C_κ -gene segment from the cosmids of the two regions (and from all our other cosmids) argues against the presence of a processed pseudogene. No other V_κ genes were

found on the cosmids of regions Chr1 and 15 or on cosmids extending these regions in the upstream direction by about 30 kb.

The finding of several hybridizing *Eco*RI fragments in digests of DNA from the chromosome 15-containing cell hybrids (Fig. 2 legend) suggests that chromosome 15 contains several copies either of the whole Chr15 region including the V_κ I gene or of only the region around the search clone used in the hybridization experiment. In the latter case, the V_κ I-gene region must have been inserted in the neighbourhood of one of the copies of this low-repetitive, chromosome-specific sequence.

Region Chr22 contains a cluster of three V_κ genes (Fig. 1). The V_κ I gene (Fig. 3) contains mutations in its start codon and in the conserved heptanucleotide sequence¹⁰⁻¹². It also carries a 7-base pair (bp) insertion in framework region 3 (FR3), which has probably been created by a duplication. Two stop codons were found in the V_κ II gene; an insertion and a deletion have to be assumed in order to obtain a good fit of the invariant amino acids¹³. Also, the V_κ III gene is a strongly diverged pseudogene. We assign it to this subgroup because it hybridizes weakly with a V_κ III probe (21-4 in ref. 5) and not at all with probes of other subgroups. Also, the invariant amino acids fit this subgroup better than the others. Peculiar T-rich sequences appear at the end of the gene and in the downstream region.

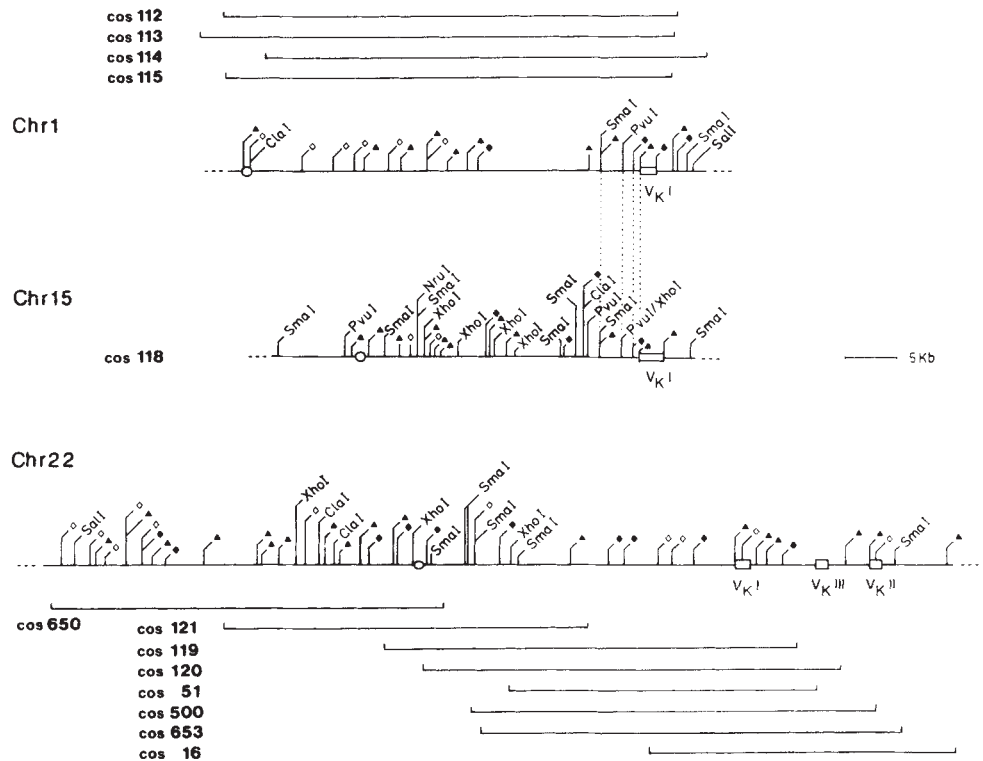
A genomic region containing a cluster of eight V_κ genes which is, at least in part, duplicated within the genome⁶ and a C_κ -gene probe were included in the present study for comparison. The localization of C_κ to chromosome 2 (ref. 1) was confirmed and the V_κ -gene region was also located on this chromosome (Fig. 2).

The possibility that the search clone and V_κ -gene regions have become associated artefactually can be excluded for Chr1 and Chr22 because these genomic regions were constructed from several independently isolated cosmids which were identical in the overlapping parts (Fig. 1). Ligation artefacts were extremely rare in the many cosmids that we have isolated from our libraries, and so it is very likely that the Chr15 structure, although based on only one cosmid, also represents a continuous genomic region.

We estimate roughly, based on counting of cloned and chromosomally located genes, that about 10% of the human V_κ genes are located outside chromosome 2. However, the proportion cannot be determined exactly for the following reasons: a fair number of our cloned V_κ genes are still not sufficiently characterized; it is difficult to establish the completeness of library screening experiments; restriction analyses of cosmids containing dispersed V_κ genes suggest that some sequences are present on the respective chromosomes in more than one copy; and finally, the size of the duplicated section of the V_κ locus on chromosome 2 (ref. 6) is not known. A different approach to the question, hybridization of V_κ -gene probes to the panel of human-rodent cell hybrid DNAs, did not yield clear results mainly because of cross-hybridization of the human V_κ genes (V_κ I, V_κ III) with rodent V_κ genes and because of the limits of sensitivity (V_κ II). On the basis of our present data, we conclude that a small but significant fraction of the human V_κ genes is dispersed outside chromosome 2.

In addition to the dispersed V_κ pseudogenes described here, some human V_κ pseudogenes have been found on chromosome 2 (see, for example, refs 4, 6), while others exist which have not yet been mapped to specific chromosomes^{3,14}. The dispersed pseudogenes are more widely diverged from the potentially functional genes than are the pseudogenes located on chromosome 2; they are perhaps no longer or less subject to the surveillance by gene conversion-like processes which occurs between adjacent or more distantly located genes of a locus (see, for example, refs 3, 4, 15). Distinct from the non-processed pseudogenes are the processed genes that arise by transcription and retrotranscription events and which are quite common among several gene families (reviewed in ref. 16). Processed human $J_\kappa C_\lambda$ (J_κ , joining; ref. 17) and C_ϵ (refs 18, 19) pseudogenes have also been found, but, notably, no processed V pseudogenes

Fig. 1 V_{κ} -gene-containing regions on several chromosomes. The restriction maps of the genomic regions are derived from the cosmids indicated in the figure. All cosmids were isolated from libraries prepared from the same DNA (ref. 4 and H.-D.P., unpublished data). Cosmids are indicated by horizontal lines, V_{κ} -gene regions by boxes, search clone regions by circles. The 5'-3' polarity of regions Chr1 and Chr15 is based on map homology (vertical dashed lines) to another genomic region (not shown) in which the V_{κ} I gene was sequenced (R. Thiebe, unpublished). The search clones (in M13; ref. 21), which were selected for the absence of repetitive sequences, are a 1.0-kb *Bgl*II/*Cla*I fragment (Chr1), a 1.4-kb *Bgl*II/*Bgl*II fragment (Chr15) and a 1.3-kb *Bam*HI/*Xho*I fragment (Chr22). Detailed maps of the subcloned regions and additional map data are reported elsewhere²²⁻²⁵ and are available on request. Nuclease cleavage sites: $\blacktriangle$, *Bgl*II; $\blacklozenge$, *Bam*HI; $\diamond$, *Kpn*I.

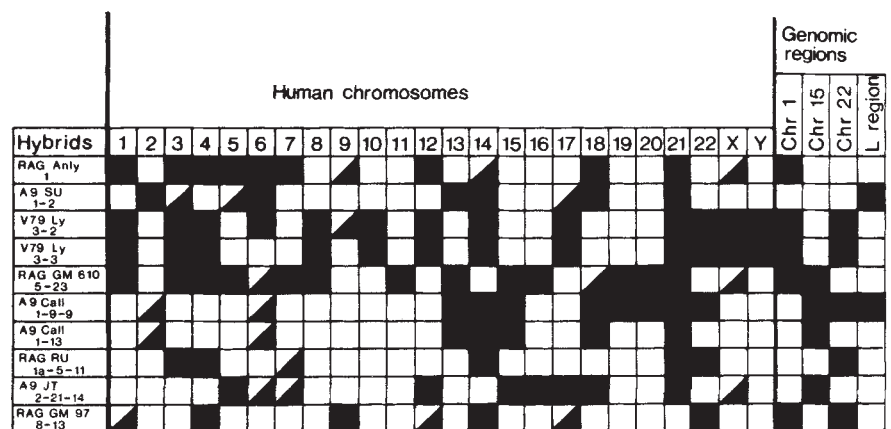


have been detected. This may reflect the fact that V_{κ} -gene segments are probably not transcribed in the germ line.

The mechanism of dispersion of V_{κ} -gene regions is unknown. For other gene families, two mechanisms in addition to retrotranscript insertion and recombinational events have been considered; these are gene conversion-like processes and the dispersion of transposon-like elements (reviewed in ref. 20). The presence of long but imperfect repeats in the flanks of several V_{κ} I genes⁴ indicates that such genes may have functioned as transposon-like elements. Also, the evolutionary history of a mixed V_{κ} I, V_{κ} II, V_{κ} III gene cluster is best discussed in terms

of intrachromosomal transposition events⁵. The presence of V_{κ} I genes on chromosomes 1 and 15 may be explained by an interchromosomal transposition, although gene conversion-like events cannot be excluded. The transposition of a V_{κ} gene cluster to chromosome 22 is difficult to explain by either mechanism. Perhaps a novel type of recombinational mechanism must be considered in this case. As the dispersed V_{κ} genes do not contribute directly to the repertoire of potentially functional V_{κ} genes, studies on the size of the V_{κ} -gene repertoire must not only count the genes but also determine their chromosomal location.

Fig. 2 Chromosomal location of V_{κ} -gene-containing regions. The subcloned fragments of regions Chr1, Chr15, Chr22 (Fig. 1) and fragment 21-1 of the region with eight V_{κ} genes (refs 4, 6; called the L region because of the occurrence of the repetitive L sequences next to the V_{κ} I genes²⁶) were self-ligated and used as hybridization probes. A panel of 27 mouse-human cell hybrids was generated by fusion of mouse RAG or A9 cells and human fibroblasts originating from various translocation carriers²⁷⁻²⁹ and characterized by isozyme and cytogenetic analyses for the presence of human chromosomes and chromosome fragments, as described previously²⁷. In addition, two human-chinese hamster cell hybrids generated by fusion of V79 cells with human lymphocytes from a male (46, XY) donor were included in the segregation analysis. Hybrid cell DNAs were digested with *Eco*RI, separated by agarose (0.8%) electrophoresis and transferred to Biotodyne A membranes (Pall Filtrationstechnik, D-6072 Dreieich). Hybridization of nick-translated probes was as in ref. 5 except that the SDS content of the solutions was 0.5% and the final wash solution contained 0.5× SSC. In these conditions, the probes did not hybridize to rodent DNAs. The *Eco*RI fragments of genomic DNA which are detected in the panels have the following lengths (in kb): Chr1, 2.1; Chr15, 6.2, 9, 12, 20, >20 (and 4 weak bands; the 9-kb fragment is derived from the V_{κ} -gene-containing region as represented in cos 118); Chr22, 3.4; L region, 8.2, ~20. All fragments detected by one of the four probes, respectively, segregated concordantly in individual hybrid clones. The segregation analysis of the fragments and human chromosomes in the panel, part of which is shown in the figure, allows the regions detected by the probes to be assigned to chromosomes 1, 15, 22 and 2, respectively. Filled spaces indicate the presence of the chromosome, half-filled spaces indicate that part of the chromosome is present, and empty spaces indicate that the chromosome was not detected by biochemical and cytogenetic analyses. Clone A9 Call 1-9-9 contains a fragment of chromosome 2 (qter-p23) and shows bands with the L-region probe 21-2. Hybrid clone RAG GM 97 8-13 retains the fragment 1pter-q12 and shows the band detected by Chr1. These findings allow us to submap the regions to parts of chromosomes 1 and 2, respectively.



a

TGTTCTATGCTAGTACTGAGATGAGCCAGCCCTGCAGCTATGCGAGCCTGCTCCTCTGCTATTAGCATGTTCCAGAGACACCCCTGCG -254

CCTGAAGACTTCTTAAGGCTGGTCACACCCGTGTCAGGAGTCAGCCCACTCAGGACACAGCAGGACGCGAGGGGCCCTCAGCTCCTGAGGCTC -11

LeuGlyLeuTrpLeuProG -154

CTGGGGCTCTGGCTGCCAGTAAGGAGGAGAACACTAGAAATTTACTCAGCCAGTGTCTCAGTACAGCTTGGCTCTTCCAGGAGGCTTCTTATAAT -4

GATTGATTATGATGATGTTTGTGTTTATGTTTCCAACTCAGTACAGAGCTGATATTCAGATGACCCAGTCTCCATCTCCCTGCTGCTATCTGATG -54

FR1

FR2

CDR1

CDR2

CDR3

FR3

FR4

FR5

FR6

FR7

FR8

FR9

FR10

FR11

FR12

FR13

FR14

FR15

FR16

FR17

FR18

FR19

FR20

FR21

FR22

FR23

FR24

FR25

FR26

FR27

FR28

FR29

FR30

FR31

FR32

FR33

FR34

FR35

FR36

FR37

FR38

FR39

FR40

FR41

FR42

FR43

FR44

FR45

FR46

FR47

FR48

FR49

FR50

FR51

FR52

FR53

FR54

FR55

FR56

FR57

FR58

FR59

FR60

FR61

FR62

FR63

FR64

FR65

FR66

FR67

FR68

FR69

FR70

FR71

FR72

FR73

FR74

FR75

FR76

FR77

FR78

FR79

FR80

FR81

FR82

FR83

FR84

FR85

FR86

FR87

FR88

FR89

FR90

FR91

FR92

FR93

FR94

FR95

FR96

FR97

FR98

FR99

FR100

FR101

FR102

FR103

FR104

FR105

FR106

FR107

FR108

FR109

FR110

FR111

FR112

FR113

FR114

FR115

FR116

FR117

FR118

FR119

FR120

FR121

FR122

FR123

FR124

FR125

FR126

FR127

FR128

FR129

FR130

FR131

FR132

FR133

FR134

FR135

FR136

FR137

FR138

FR139

FR140

FR141

FR142

FR143

FR144

FR145

FR146

FR147

FR148

FR149

FR150

FR151

FR152

FR153

FR154

FR155

FR156

FR157

FR158

FR159

FR160

FR161

FR162

FR163

FR164

FR165

FR166

FR167

FR168

FR169

FR170

FR171

FR172

FR173

FR174

FR175

FR176

FR177

FR178

FR179

FR180

FR181

FR182

FR183

FR184

FR185

FR186

FR187

FR188

FR189

FR190

FR191

FR192

FR193

FR194

FR195

FR196

FR197

FR198

FR199

FR200

FR201

FR202

FR203

FR204

FR205

FR206

FR207

FR208

FR209

FR210

FR211

FR212

FR213

FR214

FR215

FR216

FR217

FR218

FR219

FR220

FR221

FR222

FR223

FR224

FR225

FR226

FR227

FR228

FR229

FR230

FR231

FR232

FR233

FR234

FR235

FR236

FR237

FR238

FR239

FR240

FR241

FR242

FR243

FR244

FR245

FR246

FR247

FR248

FR249

FR250

FR251

FR252

FR253

FR254

FR255

FR256

FR257

FR258

FR259

FR260

FR261

FR262

FR263

FR264

FR265

FR266

FR267

FR268

FR269

FR270

FR271

FR272

FR273

FR274

FR275

FR276

FR277

FR278

FR279

FR280

FR281

FR282

FR283

FR284

FR285

FR286

FR287

FR288

FR289

FR290

FR291

FR292

FR293

FR294

FR295

FR296

FR297

FR298

FR299

FR300

FR301

FR302

FR303

FR304

FR305

FR306

FR307

FR308

FR309

FR310

FR311

FR312

FR313

FR314

FR315

FR316

FR317

FR318

FR319

FR320

FR321

FR322

FR323

FR324

FR325

FR326

FR327

FR328

FR329

FR330

FR331

FR332

FR333

FR334

FR335

FR336

FR337

FR338

FR339

FR340

FR341

FR342

FR343

FR344

FR345

FR346

FR347

FR348

FR349

FR350

FR351

FR352

FR353

FR354

FR355

FR356

FR357

FR358

FR359

FR360

FR361

FR362

FR363

FR364

FR365

FR366

FR367

FR368

FR369

FR370

FR371

FR372

FR373

FR374

FR375

FR376

FR377

FR378

FR379

FR380

FR381

FR382

FR383

FR384

FR385

FR386

FR387

FR388

FR389

FR390

FR391

FR392

FR393

FR394

FR395

FR396

FR397

FR398

FR399

FR400

FR401

FR402

FR403

FR404

FR405

FR406

FR407

FR408

FR409

FR410

FR411

FR412

FR413

FR414

FR415

FR416

FR417

FR418

FR419

FR420

FR421

FR422

FR423

FR424

FR425

FR426

FR427

FR428

FR429

FR430

FR431

FR432

FR433

FR434

FR435

FR436

FR437

FR438

FR439

FR440

FR441

FR442

FR443

FR444

FR445

FR446

FR447

FR448

FR449

FR450

FR451

FR452

FR453

FR454

FR455

FR456

FR457

FR458

FR459

FR460

FR461

FR462

FR463

FR464

FR465

FR466

FR467

FR468

FR469

FR470

FR471

FR472

FR473

FR474

FR475

FR476

FR477

FR478

FR479

FR480

FR481

FR482

FR483

FR484

FR485

FR486

FR487

FR488

FR489

FR490

FR491

FR492

FR493

FR494

FR495

FR496

FR497

FR498

FR499

FR500

FR501

FR502

FR503

FR504

FR505

FR506

FR507

FR508

FR509

FR510

FR511

FR512

FR513

FR514

FR515

FR516

FR517

FR518

FR519

FR520

FR521

FR522

FR523

FR524

FR525

FR526

FR527

FR528

FR529

FR530

FR531

FR532

FR533

FR534

FR535

FR536

FR537

FR538

FR539

FR540

FR541

FR542

FR543

FR544

FR545

FR546

FR547

FR548

FR549

FR550

FR551

FR552

FR553

FR554

FR555

FR556

FR557

FR558

FR559

FR560

FR561

FR562

FR563

FR564

FR565

FR566

FR567

FR568

FR569

FR570

FR571

FR572

FR573

FR574

FR575

FR576

FR577

FR578

FR579

FR580

FR581

FR582

FR583

FR584

FR585

FR586

FR587

FR588

FR589

FR590

FR591

FR592

FR593

FR594

FR595

FR596

FR597

FR598

FR599

FR600

FR601

FR602

FR603

FR604

FR605

FR606

FR607

FR608

FR609

FR610

FR611

FR612

FR613

FR614

FR615

FR616

FR617

FR618

FR619

FR620

FR621

FR622

FR623

FR624

FR625

FR626

FR627

FR628

FR629

FR630

FR631

FR632

FR633

FR634

FR635

FR636

FR637

FR638

FR639

FR640

FR641

FR642

FR643

FR644

FR645

FR646

FR647

FR648

FR649

FR650

FR651

FR652

FR653

FR654

FR655

FR656

FR657

FR658

FR659

FR660

FR661

FR662

FR663

FR664

FR665

FR666

FR667

FR668

FR669

FR670

FR671

FR672

FR673

FR674

FR675

FR676

FR677

FR678

FR679

FR680

FR681

FR682

FR683

FR684

FR685

FR686

FR687

FR688

FR689

FR690

FR691

FR692

FR693

FR694

FR695

FR696

FR697

FR698

FR699

FR700

FR701

FR702

FR703

FR704

FR705

FR706

FR707

FR708

FR709

FR710

FR711

FR712

FR713

FR714

FR715

FR716

FR717

FR718

FR719

FR720

FR721

FR722

FR723

FR724

FR725

FR726

FR727

FR728

FR729

FR730

FR731

FR732

FR733

FR734

FR735

FR736

FR737

FR738

FR739

FR740

FR741

FR742

FR743

FR744

FR745

FR746

FR747

FR748

FR749

FR750

FR751

FR752

FR753

FR754

FR755

FR756

FR757

FR758

FR759

FR760

FR761

FR762

FR763

FR764

FR765

FR766

FR767

FR768

FR769

FR770

FR771

FR772

FR773

FR774

FR775

FR776

FR777

FR778

FR779

FR780

FR781

FR782

FR783

FR784

FR785

FR786

FR787

FR788

FR789

FR790

FR791

FR792

FR793

FR794

FR795

FR796

FR797

FR798

FR799

FR800

FR801

FR802

FR803

FR804

FR805

FR806

FR807

FR808

FR809

FR810

FR811

FR812

FR813

FR814

FR815

FR816

FR817

FR818

FR819

FR820

FR821

FR822

FR823

FR824

FR825

FR826

FR827

FR828

FR829

FR830

FR831

FR832

FR833

FR834

FR835

FR836

FR837

FR838

FR839

FR840

FR841

FR842

FR843

FR844

FR845

FR846

FR847

FR848

FR849

FR850

FR851

FR852

FR853

FR854

FR855

FR856

FR857

FR858

FR859

FR860

FR861

FR862

FR863

FR864

FR865

FR866

FR867

FR868

FR869

FR870

FR871

FR872

FR873

FR874

FR875

FR876

FR877

FR878

FR879

FR880

FR881

FR882

FR883

FR884

FR885

FR886

FR887

FR888

FR889

FR890

FR891

FR892

FR893

FR894

FR895

FR896

FR897

FR898

FR899

FR900

FR901

FR902

FR903

FR904

FR905

FR906

FR907

FR908

FR909

FR910

FR911

FR912

FR913

FR914

FR915

FR916

FR917

FR918

FR919

FR920

FR921

FR922

FR923

FR924

FR925

FR926

FR927

FR928

FR929

FR930

FR931

FR932

FR933

FR934

FR935

FR936

FR937

FR938

FR939

FR940

FR941

FR942

FR943

FR944

FR945

FR946

FR947

FR948

FR949

FR950

FR951

FR952

FR953

FR954

FR955

FR956

FR957

FR958

FR959

FR960

FR961

FR962

FR963

FR964

FR965

FR966

FR967

FR968

FR969

FR970

FR971

FR972

FR973

FR974

FR975

FR976

FR977

FR978

FR979

FR980

FR981

FR982

FR983

FR984

FR985

FR986

FR987

FR988

FR989

FR990

FR991

FR992

FR993

FR994

FR995

FR996

FR997

FR998

FR999

FR1000

b

AGATCTTGAAGATAGAGTTCCTGACTGCTAGTAATTTGTCATTCATTTAGAAATCTACTTTTGATGATATAAATCTAACTTGAAGAAATACGTAA -96

CTGTAAATGAATATCATAAGGGAATCATGAAAGTGTCTCATAGTGTGTCTATATAAECTTACACTTCTTTTATGTTATTTACAGGCTCCAGTGGGATAT -2

FR1

CDR1

FR2

FR3

FR4

FR5

FR6

FR7

FR8

FR9

FR10

FR11

FR12

FR13

FR14

FR15

FR16

FR17

FR18

FR19

FR20

FR21

FR22

FR23

FR24

FR25

FR26

FR27

FR28

FR29

FR30

FR31

FR32

FR33

FR34

FR35

FR36

FR37

FR38

FR39

FR40

FR41

FR42

FR43

FR44

FR45

FR46

FR47

FR48

FR49

FR50

FR51

FR52

FR53

FR54

FR55

FR56

FR57

FR58

FR59

FR60

FR61

FR62

FR63

FR64

FR65

FR66

FR67

FR68

FR69

FR70

FR71

FR72

FR73

FR74

FR75

FR76

FR77

FR78

FR79

FR80

FR81

FR82

FR83

FR84

FR85

FR86

FR87

FR88

FR89

FR90

FR91

FR92

FR93

FR94

FR95

FR96

FR97

FR98

FR99

FR100

FR101

FR102

FR103

FR104

FR105

FR106

FR107

FR108

FR109

FR110

FR111

FR112

FR113

FR114

FR115

FR116

FR117

FR118

FR119

FR120

FR121

FR122

FR123

FR124

FR125

FR126

FR127

FR128

FR129

FR130

FR131

FR132

FR133

FR134

FR135

FR136

FR137

FR138

FR139

FR140

FR141

FR142

FR143

FR144

FR145

FR146

FR147

FR148

FR149

FR150

FR151

FR152

FR153

FR154

FR155

FR156

FR157

FR158

FR159

FR160

FR161

FR162

FR163

FR164

FR165

FR166

FR167

FR168

FR169

FR170

FR171

FR172

FR173

FR174

FR175

FR176

FR177

FR178

FR179

FR180

FR181

FR182

FR183

FR184

FR185

FR186

FR187

FR188

FR189

FR190

FR191

FR192

FR193

FR194

FR195

FR19

Increased phosphorylation of ribosomal protein S6 following microinjection of insulin receptor-kinase into *Xenopus* oocytes

James L. Maller*, Linda J. Pike†‡, Gary R. Freidenberg§, Renzo Cordera§, Bradley J. Stith*, Jerrold M. Olefsky§ & Edwin G. Krebs†

* Department of Pharmacology, University of Colorado School of Medicine, Denver, Colorado 80262, USA

† Howard Hughes Medical Institute, University of Washington, Seattle, Washington 98195, USA

§ Department of Medicine, University of California, San Diego, La Jolla, California 92093, USA

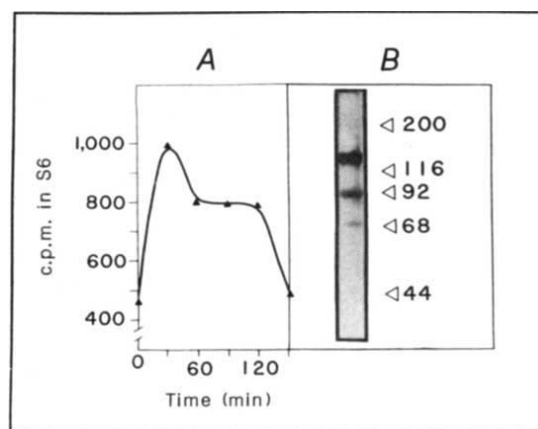


Fig. 1 S6 phosphorylation following microinjection of insulin-receptor kinase into *Xenopus* oocytes. **A**, Time course of increase in S6 phosphorylation. Insulin receptors purified by affinity chromatography ($100 \mu\text{g ml}^{-1}$) and incubated with 100 nM insulin were microinjected into each of 25 oocytes preinjected with 50 nl of $^{32}\text{P}_i$ (200 mCi ml^{-1}). Control (0 time) oocytes were injected with 100 nM insulin, 1 mg ml^{-1} bovine serum albumin (BSA) in the receptor kinase buffer. At the indicated times ribosomes were isolated and subjected to SDS-gel electrophoresis. The S6 band was identified by autoradiography, excised and counted. **B**, Silver-stained gel of the purified receptor preparation.

Methods. Receptors were purified from a fresh full-term human placenta by a modification of the method reported by Petruzelli *et al.*²⁶. Briefly, the placenta was washed in phosphate-buffered saline, diced and homogenized in a Polytron homogenizer for 1 min. Following centrifugation at $600g$ for 5 min, the supernatant was centrifuged at $50,000g$ for 2 h and the membrane pellet resuspended in 25 mM HEPES pH 7.5, 0.25 M sucrose, 1 mM MgCl_2 , 0.1 mM phenylmethylsulphonyl fluoride, 100 mg ml^{-1} bacitracin and 1% Triton X-100. After 1 h at 4°C , the membranes were re-centrifuged at $50,000g$ for 2 h and the supernatant was diluted fivefold in 25 mM HEPES, 110 mM NaCl, 3 mM KCl, 0.9 mM MgSO_4 , 1.4 mM CaCl_2 , 2 mM EDTA, 0.05% Triton X-100 and applied to a 10-ml column of wheat-germ agglutinin-agarose (Vector Laboratories). After extensive washing of the column with the diluting buffer, the receptor was eluted at room temperature with buffer containing 0.3 M N -acetylglucosamine. Fractions were collected in ice-cold tubes and those containing receptors were identified on the basis of specific insulin binding and autophosphorylation of a $95,000\text{-M}$ component in the presence of insulin. The pertinent fractions were pooled, dialysed against 25 mM HEPES pH 7.4, 5 mM NaH_2PO_4 , 30 mM NaCl, 0.2% Triton X-100, 10 mM imidazole, 50% ethylene glycol, and stored at -30°C . A typical preparation had an insulin binding activity of 30 pmol mg^{-1} , and a specific activity for phosphorylation of a tyrosine-containing polymer (Glu-Tyr, 1:4) of $38 \text{ pmol per min per mg}$. For affinity purification, a preparation purified through wheat-germ agglutinin chromatography was adsorbed to and eluted from an insulin-Sepharose column by a modification of the method of Fujita-Yamaguchi *et al.*²⁷. The purified receptor was concentrated by adsorption on a small column of DEAE-cellulose and eluted with a small volume of 40 mM imidazole pH 7.2, 10% glycerol, 250 mM NaCl, 0.2% Triton X-100 (ref. 14). The receptor had a specific activity for insulin binding of $3,850 \text{ pmol mg}^{-1}$ and for phosphorylation of $300 \text{ nmol per min per mg}$ using as substrate a synthetic peptide corresponding to the autophosphorylation site of pp60^{src} (ref. 14). The receptor was stored in small aliquots at -70°C . Incubation of the preparation with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ plus Mn^{2+} and Mg^{2+} resulted in the phosphorylation of a $95,000\text{-M}$ protein and this phosphorylated material could be immunoprecipitated with antiserum to the insulin receptor (antiserum B9 kindly provided by Dr C. Ron Kahn, Joslin Diabetes Center, Harvard Medical School). For microinjection experiments, the receptor was activated by addition of insulin to 10^{-7} M followed by incubation at room temperature for 15 min. Control incubations were with insulin alone in the kinase buffer containing 1 mg ml^{-1} BSA. After the 15-min incubation, the preparations were kept on ice before being loaded into the microinjection needle. Experiments demonstrated that this procedure activated the tyrosine kinase activity of the receptor as judged by autophosphorylation. Procedures for isolating oocytes and ribosomes are described elsewhere⁹. Silver staining of polyacrylamide gels was carried out using a Bio-Rad silver-stain kit.

The protein products of several transforming retroviruses as well as the receptors for several hormones and growth factors, including insulin, have been shown to possess a protein kinase activity *in vitro* specific for tyrosine residues in protein substrates, including themselves (see ref. 1 for a review). In the case of pp60^{src} and the insulin receptor, autophosphorylation activates the tyrosine kinase activity towards exogenous substrates²⁻⁴. Experiments indicate that, *in vivo*, many of these viruses or growth factors induce an increase in cellular phosphotyrosine, as well as an increase in the phosphorylation of serine residues on proteins, including ribosomal protein S6. It seems likely that some of the effects of insulin might be mediated by phosphorylation of intracellular substrates by its receptor. As the β subunit of the receptor is a transmembrane protein⁵, such phosphorylation could occur either while the receptor is still in the membrane or after its internalization. In various cell systems, internalized receptors are degraded, reshuttled back to the plasmalemma or maintained in a separate compartment before reinsertion in the membrane^{6,7}; shuttling of the insulin receptor could provide the opportunity for it to phosphorylate various intracellular components as part of its mechanism of signal transduction. To approach directly the question of whether the receptor can elicit a signal while acting at an intracellular location, we have microinjected *Xenopus* oocytes with the insulin receptor kinase. The results indicate that an S6 protein-serine kinase is stimulated or an S6 protein-serine phosphatase inhibited by the activity of the insulin receptor, supporting the concept that the insulin receptor acting within the cell can elicit a biological response.

Oocytes possess insulin receptors⁸ and respond to insulin by increased intracellular pH , increased phosphorylation of proteins, including ribosomal protein S6, and increased protein synthesis⁹⁻¹¹. The phosphorylation of serine in S6 is increased in oocytes following the microinjection of purified pp60^{src} , the protein-tyrosine kinase responsible for transformation by Rous sarcoma virus¹² or the purified protein-tyrosine kinase involved in transformation by Abelson murine leukaemia virus¹³. *Xenopus* oocytes must therefore possess the regulatory pathways utilized by exogenous protein-tyrosine kinases to cause S6 phosphorylation. To obtain further evidence for this, the phosphorylation of S6 was examined after microinjection of insulin receptors into oocytes. The receptor preparation eluted from insulin-Sepharose was highly purified as judged by gel electrophoresis and silver staining (Fig. 1B). (However, this purified form of the solubilized insulin receptor does not necessarily represent the state of the receptor after internalization in a target cell.) Following microinjection of receptors with bound insulin, the phosphorylation of S6 increased rapidly to about threefold that of control oocytes (Fig. 1A). The response is unlikely to be due to leakage of insulin from the injected oocyte because all data

are expressed relative to controls injected with insulin in the receptor kinase buffer. Maximal increases in S6 phosphorylation were observed earlier than has been reported for oocytes treated with hormones⁹, and by 3 h after injection, S6 phosphorylation had returned to the basal level. Phosphoamino-acid analysis of S6 from receptor-injected oocytes revealed exclusively phosphoserine while incubation of 40S subunits with the receptor kinase and $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ *in vitro* produced no S6 phosphorylation (data not shown).

‡ Present address: Washington University, Department of Biological Chemistry, Howard Hughes Medical Institute, 660 South Euclid Avenue, St Louis, Missouri 63110, USA.

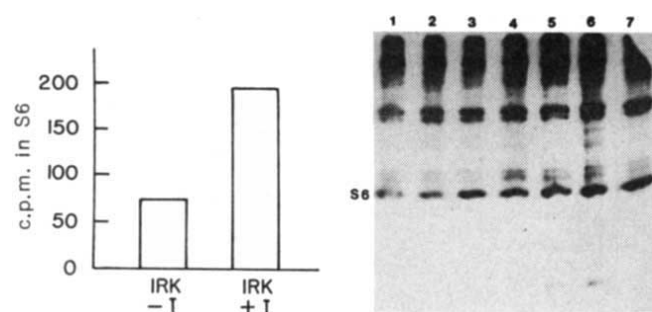


Fig. 2 Insulin stimulation and dose dependence of S6 phosphorylation by injected receptor kinase. **A**, Insulin stimulation of S6 phosphorylation by injected receptor kinase. Insulin receptors purified through chromatography on wheat-germ agglutinin agarose were microinjected with or without 100 nM insulin pretreatment into each of 50 oocytes. For insulin pretreatment, the receptor preparation was incubated for 15 min at 22 °C with 100 nM insulin, 1 mg ml⁻¹ BSA or the BSA vehicle only. S6 phosphorylation was determined as described in Fig. 1 legend except that oocytes were incubated for 4 h with 0.5 mCi ml⁻¹ ³²P before injection of the receptor instead of being microinjected with ³²P. The use of incubation labelling accounts for the lower level of radioactivity in S6 compared with Fig. 1. The background c.p.m. in S6 (40 c.p.m.) in oocytes injected with buffer containing 100 nM insulin have been subtracted from the values shown. IRK, insulin receptor kinase; I, insulin. **B**, Dose dependence for S6 phosphorylation by injected receptor kinase. Receptors purified through affinity chromatography on insulin-Sepharose were diluted in the kinase buffer (Fig. 1) containing 100 nM insulin plus 1 mg ml⁻¹ BSA. A 60- μ l aliquot of each dilution was injected into each of 30 oocytes and the level of S6 phosphorylation determined by gel electrophoresis and autoradiography as described in Fig. 1 legend. The concentration of insulin receptor injected ($\mu\text{g ml}^{-1}$) was: lane 1, 1; lane 2, 2; lane 3, 5; lane 4, 10; lane 5, 20; lane 6, 50; lane 7, 100.

The insulin receptor possesses appreciable basal protein-tyrosine kinase activity. As Fig. 2A shows, the receptor kinase was able to cause some increase in S6 phosphorylation in the absence of bound insulin, but its activity was doubled after pretreatment with insulin. This degree of stimulation is comparable to the degree of insulin stimulation of the phosphorylation of a synthetic peptide substrate *in vivo*¹⁴. The increase in S6 phosphorylation was dose dependent (Fig. 2B); receptor preparations diluted more than 100-fold were unable to cause S6 phosphorylation, while maximal change in S6 phosphorylation was induced with the most concentrated form of the receptor.

Although the response to microinjected receptor was very rapid and the receptor was not injected as an endocytotic vesicle, it was important to evaluate the possibility that injected insulin receptors might be rapidly reinserted in the plasma membrane after microinjection. Several types of experiment were carried out to assess this possibility. Previous studies in adipocytes and hepatocytes have demonstrated that insulin receptors photolabelled with ¹²⁵I-NAPA-DP-insulin (2 nitro, 4-azidophenyl-acetyl des-Phe-B1-insulin) can be used to monitor recycling of insulin receptors in target cells^{6,15-18}. Such recycling is measured by the loss or reappearance of trypsin-sensitive radioactivity on the intact cell surface as well as by the appearance of degraded forms of immunoprecipitable receptor which migrate on polyacrylamide gels with discrete relative molecular masses (M_s)¹⁶⁻¹⁸. Table 1 shows that more than 90% of the injected photolabelled receptor was resistant to trypsin treatment of intact oocytes when S6 phosphorylation was maximal. Moreover, immunoprecipitation of receptors from injected oocytes revealed no degraded forms (data not shown). When, in other experiments, injected oocytes were homogenized and fractionated by differential centrifugation, less than 2% of the injected receptor was present in the plasma membrane fraction (Table 1). These results make it very unlikely that increased S6 phosphorylation is a result of the action of injected receptors that are reinserted in the plasma membrane.

In vivo, S6 exists in multiply-phosphorylated forms containing up to 5 mol phosphate per mol S6. Using a two-dimensional gel system designed for resolution of basic ribosomal proteins, S6 and five of its derivatives corresponding to increasing numbers

Table 1 Effect of trypsin treatment on insulin receptors in injected oocytes

Source	-Trypsin	+Trypsin
Oocytes	485	450
Oocytes	352	339
Oocytes	407	341
Immunoprecipitate	340	338

Fraction	% Total c.p.m.
P1	6.37 $\pm$ 0.16
P2	1.88 $\pm$ 0.42
P3	6.40 $\pm$ 1.03
S3	85.0 $\pm$ 0.58

NAPA-DP-insulin was prepared as described previously¹⁶. Oocytes (25 per group) were microinjected with ¹²⁵I-NAPA-DP-labelled insulin receptors, incubated for 30 min at 24 °C, and some groups were then exposed to trypsin (200 $\mu\text{g ml}^{-1}$ final concentration) for 30 min at 4 °C followed by extensive washing and addition of 2 mg ml⁻¹ soybean trypsin inhibitor. In each of three experiments, injected oocytes were counted in a γ counter before and after trypsin treatment. From a total of 52 trypsin-treated and 52 trypsin-untreated oocytes from two experiments, solubilized material was extracted in a solution containing 2% Triton X-100, 10 mM benzimidazole, 2 mM phenylmethylsulphonyl fluoride, 1,000 kU ml⁻¹ aprotinin and 2 mg ml⁻¹ bacitracin, pH 7.4. The labelled insulin receptors were immunoprecipitated by incubation of the soluble extract with anti-insulin-receptor antibody for 16 h at 4 °C followed by a 1-h incubation with protein A. The numbers in the upper portion of the table refer to the c.p.m. with and without trypsin treatment in intact washed oocytes and in immunoprecipitates of receptors from oocytes. In the lower portion, using a photolabelled receptor preparation of higher specific activity, the percentage of injected receptor c.p.m. in different oocyte fractions is presented ($\pm$ s.e.m., $n=4$). P1 is material sedimenting at 1,000g for 15 min, P2 sedimenting at 10,000g for 20 min, P3 sedimenting at 165,000g for 20 min, and S3 material soluble after centrifugation at 165,000g. Fraction P2 has been demonstrated previously to contain nearly all the plasma membrane on the basis of membrane-associated enzyme activities²⁵.

of phosphate groups were visualized¹⁰. Each derivative contains a unique phosphopeptide relative to the preceding derivative, which suggests that phosphorylation of S6 is ordered¹⁹. Consequently, the distribution of ³²P_i among the derivatives of S6 gives an indication of its phosphorylation state independent of total ³²P_i content. In control oocytes injected with insulin (1 μM) alone in buffer, after 90 min most of the phosphorylated S6 was in derivative *a* (1 mol P_i per mol S6), with a small amount of *b* derivative detectable. This is the same pattern as seen in uninjected oocytes. In oocytes injected with insulin receptor kinase, derivative *b* was the predominant labelled species, with significant amounts of the more highly phosphorylated derivatives *c* and *d* visible (Fig. 2B). In order to visualize the derivatives, the autoradiograph in Fig. 2A was exposed for a considerably longer period than in *B*. Hence the increase in S6 phosphorylation in response to the injected insulin receptor is not apparent in Fig. 3. Following external application of insulin (Fig. 2C) or progesterone (Fig. 2D) until the time of germinal vesicle breakdown (5 h), all the S6 molecules became maximally phosphorylated, as reported previously^{9,20}. These results indicate that phosphorylation of S6 in response to insulin receptor kinase reflects the increasing phosphorylation of individual S6 molecules as well as an increase in the number of ribosomes labelled in oocytes.

The experiments reported here support the concept that the insulin receptor can elicit a biological response while acting at an intracellular location, possibly including the inner surface of the plasma membrane. Although the receptors caused an increase in S6 phosphorylation in the absence of insulin pretreatment (Fig. 2), the fact that insulin stimulated peptide phosphorylation and S6 phosphorylation to the same extent (Fig. 2 and ref. 14) suggests that the protein kinase activity of the receptor

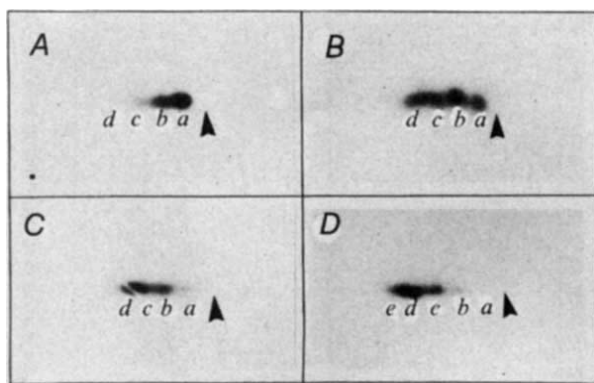


Fig. 3 Two-dimensional gel electrophoretic analysis of S6. ^{32}P -labelled oocytes were microinjected with buffer containing $1\text{ }\mu\text{M}$ insulin (A) or with insulin receptors eluted from wheat-germ agglutinin-agarose and incubated with 100 nM insulin (B), or treated with external insulin ($1\text{ }\mu\text{M}$, C) or external progesterone ($10\text{ }\mu\text{M}$, D). The arrow marks the position of unphosphorylated S6 and the letters a–e refer to S6 derivatives containing increasing numbers of phosphate groups.

Methods. Ribosomal proteins for two-dimensional gel electrophoresis were extracted as described previously²⁰. ^{32}P -labelled samples for two-dimensional gel electrophoresis were mixed with unlabelled carrier *Xenopus* oocyte ribosomal protein (unphosphorylated S6) and *Xenopus* eggs (phosphorylated S6). Ribosomal species corresponding to the unphosphorylated form (indicated by an arrow) and derivatives corresponding to increasing numbers of phosphate groups (designated a–e for 1–5 mol phosphate)^{10,13,21} were assigned on the basis of the Coomassie blue staining pattern of the co-electrophoresed ribosomal carrier protein.

is responsible for its ability to cause S6 phosphorylation. The fact that S6 is phosphorylated at serine residues while the insulin receptor is a protein-tyrosine kinase, coupled with the finding that *in vitro* the receptor alone was unable to phosphorylate 40S subunits, implies that the activity of a protein-serine kinase or phosphatase may be regulated directly or indirectly by tyrosine phosphorylation. However, we cannot exclude the possibility that some activity of the receptor other than tyrosine phosphorylation is involved in stimulating S6 phosphorylation.

We examined S6 phosphorylation in these studies because it is stimulated by external administration of insulin to oocytes and other cells^{9,10,21–23}. The exact function of S6 phosphorylation in protein synthesis is not known, although recent evidence from cultured cells indicates that ribosomes containing maximally phosphorylated S6 are preferentially utilized for polyribosome formation during serum or hormonal stimulation of protein synthesis²¹. Hence, the insulin-induced phosphorylated S6 may be related to the stimulatory effect of this hormone on protein synthesis in the oocyte¹⁰. In addition to S6 phosphorylation, other insulin effects commonly observed in target cells have been examined in oocytes, including elevation of intracellular pH due to Na/H exchange at the plasma membrane and activation of glycogen synthase, but only intracellular pH was affected by external application of insulin^{9,10}. Neither of these activities was affected by microinjection of the insulin receptor kinase (data not shown), which suggests that the mere presence of insulin-receptor kinase inside the cell is insufficient to obtain a complete insulin response. However, in its solubilized form the microinjected receptor kinase may not be accessible to all the cellular compartments necessary to induce the entire set of responses to insulin.

The present results clearly demonstrate that the insulin receptor kinase can elicit at least one insulin effect while acting at an intracellular location. Sequence analysis of the human insulin receptor gene has demonstrated a significant degree of homology with the *src* family of oncogenes⁵, and other studies show that pp60^{v-src} and the epidermal growth factor receptor have overlapping substrate specificities *in vitro* with the insulin-receptor

kinase, while antisera to pp60^{v-src} are able to immunoprecipitate the insulin-receptor kinase²⁴. Since pp60^{v-src} is able to cause S6 phosphorylation in oocytes, it is possible that the ability of the insulin receptor to cause S6 phosphorylation in oocytes reflects an overlapping substrate specificity with oncogene protein kinases. The ability of three different protein-tyrosine kinases to cause S6 phosphorylation after injection into oocytes clearly provides a system for investigating whether a common substrate is phosphorylated to increase S6 phosphorylation. Analysis of oocyte substrates for protein-tyrosine kinases and of oocyte S6 protein kinases should elucidate the importance of tyrosine phosphorylation in transduction of the insulin signal and the action of insulin-receptor kinase inside cells.

We thank Diana Smith for assistance with the two-dimensional gel analysis and Eleanor Erikson for a critical reading of the manuscript. This work was supported by grants from the NIH, the Howard Hughes Medical Institute and the Juvenile Diabetes Foundation. J.L.M. is an Established Investigator of the American Heart Association. L.J.P. is an Investigator and E.G.K. a Senior Investigator, Howard Hughes Medical Institute.

Received 9 September 1985; accepted 5 February 1986.

1. Sefton, B. M. & Hunter, T. *Adv. Cyclic Nucleotide Res.* **18**, 195–226 (1984).
2. Rosen, O. M., Herrera, R., Olowe, Y., Petruzzelli, L. M. & Cobb, M. H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3237–3240 (1983).
3. Yu, K.-T. & Czech, M. P. *J. biol. Chem.* **259**, 5277–5286 (1984).
4. Purchio, A. F., Wells, S. K. & Collett, M. S. *Molec. cell. Biol.* **3**, 1589–1597 (1983).
5. Ullrich, A. *et al. Nature* **313**, 756–761 (1985).
6. Heidenreich, K. A. & Olefsky, J. M. in *Molecular Basis of Insulin Action* (ed. Czech, M. P.) 45–65 (Plenum, New York, 1985).
7. Simpson, I. A., Hedro, J. A. & Cushman, S. W. *Diabetes* **33**, 13–18 (1984).
8. Maller, J. L. & Koontz, J. W. *Dev. Biol.* **85**, 309–316 (1981).
9. Stith, P. J. & Maller, J. L. *Dev. Biol.* **102**, 72–89 (1984).
10. Stith, B. J. & Maller, J. L. *Dev. Biol.* **107**, 460–469 (1985).
11. Maller, J. L. & Smith, D. S. *Dev. Biol.* **109**, 150–156 (1985).
12. Spivack, J. G., Erikson, R. L. & Maller, J. L. *Molec. cell. Biol.* **4**, 1631–1634 (1984).
13. Maller, J. L., Foulkes, J. G., Erikson, E. & Baltimore, D. *Proc. natn. Acad. Sci. U.S.A.* **82**, 272–276 (1985).
14. Pike, L. J., Eakes, A. T. & Krebs, E. G. *J. biol. Chem.* **261** (in the press).
15. Brandenburg, D., Draconescu, C., Saunders, D. & Thamm, P. *Nature* **286**, 821–822 (1980).
16. Heidenreich, K., Berhanu, P., Brandenburg, D. & Olefsky, J. M. *Diabetes* **32**, 1001–1009 (1983).
17. Fehlmann, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 5921–5925 (1982).
18. Fehlmann, M. *et al. J. Cell Biol.* **93**, 82–87 (1982).
19. Martin-Perez, J. & Thomas, G. *Proc. natn. Acad. Sci. U.S.A.* **80**, 926–930 (1983).
20. Nielsen, P. J., Thomas, G. & Maller, J. L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2937–2941 (1982).
21. Thomas, G., Martin-Perez, J., Siegmund, M. & Otto, A. M. *Cell* **30**, 235–242 (1982).
22. Wettenhall, R. E. H., Cohen, P., Caudwell, B. & Holland, R. *FEBS Lett.* **148**, 207–213 (1982).
23. Smith, C. J., Wejksnora, P. J., Warner, J. R., Rubin, C. S. & Rosen, O. M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2725–2729 (1979).
24. Perrotti, N. *et al. Science* **227**, 761–763 (1985).
25. Finidori, J., Hanoune, J. & Baulieu, E. E. *Molec. cell. Endocr.* **28**, 211–227 (1982).
26. Petruzzelli, L. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6792–6796 (1982).
27. Fujita-Yamaguchi, Y., Choi, S., Sakamoto, Y. & Itakura, K. *J. biol. Chem.* **258**, 5045–5049 (1983).

Ca²⁺ release from endoplasmic reticulum is mediated by a guanine nucleotide regulatory mechanism

Donald L. Gill, Teruko Ueda, Sheau-Huei Chueh & Mark W. Noel

Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, Maryland 21201, USA

Ca²⁺ accumulation and release from intracellular organelles is important for Ca²⁺-signalling events within cells^{1,2}. In a variety of cell types, the active Ca²⁺-pumping properties of endoplasmic reticulum (ER) have been directly studied using chemically permeabilized cells^{3–6}. The same preparations have been extensively used to study Ca²⁺ release from ER, in particular, release mediated by the intracellular messenger inositol 1,4,5-trisphosphate (InsP₃)^{1,2,5,7–12}. So far, these studies and others using microsomal membrane fractions^{2,11,13–15} have revealed few mechanistic details of Ca²⁺ release from ER, although a recent report¹⁶ indicated that

InsP₃-mediated Ca²⁺ release from liver microsomes may be dependent on GTP. In contrast to the latter report, we describe here the direct activation of a specific and sensitive guanine nucleotide regulatory mechanism mediating a substantial release of Ca²⁺ from the ER of cells of the neuronal cell line N1E-115. These data indicate the operation of a major new Ca²⁺ gating mechanism in ER which is specifically activated by GTP, deactivated by GDP, and which appears to involve a GTP hydrolytic cycle.

Recent detailed studies⁶ using N1E-115 neuroblastoma cells permeabilized by brief treatment with 0.005% saponin, have revealed that Ca²⁺ is accumulated within ER via a high-affinity (ATP+Mg²⁺)-dependent Ca²⁺ pump, which is distinct from that established in earlier studies as functioning in the neural plasma membrane¹⁷⁻¹⁹. Under approximately physiological intracellular conditions (cytosolic free Ca²⁺ (0.1 μM), K⁺, Na⁺, Mg²⁺ and pH), the addition of 10 μM GTP induces a large and rapid release of Ca²⁺. Figure 1 shows the remarkable effectiveness of GTP on Ca²⁺ release from within N1E-115 cells. In these experiments, cells removed from culture dishes, permeabilized and equilibrated with intracellular medium, were loaded with ⁴⁵Ca²⁺ to equilibrium for 4 min via operation of the ATP-dependent Ca²⁺ pump, as described previously⁶. Addition of 10 μM GTP caused a rapid release of over 50% of the total Ca²⁺ accumulated within cells, the effect being almost complete within 20 s (Fig. 1a). Other nucleoside triphosphates (ITP, ATP and CTP) added under identical conditions were ineffective. Moreover, similar experiments revealed that guanosine, GMP, 3',5'-cyclic GMP, 2',3'-cyclic GMP and UTP have no effect at concentrations up to 100 μM. As shown in Fig. 1a, GTP-induced Ca²⁺ release was as rapid as that mediated by the Ca²⁺ ionophore A23187. However, whereas the ionophore released 85–95% of the total accumulated Ca²⁺ from permeabilized cells, the effect of GTP was always partial, consistently releasing 65–75% of ionophore-releasable Ca²⁺. GTP had no effect on either the initial rate or affinity of the (ATP+Mg²⁺)-dependent Ca²⁺ pump within permeabilized N1E-115 cells.

Ca²⁺ release from permeabilized cells is highly sensitive to GTP. Thus, half-maximal activation of release occurred at ~0.8 μM GTP, the effect being maximal at 10 μM (Fig. 1b). As the incubation mixtures all contained 1 mM ATP (required for Ca²⁺ uptake), the effect of GTP is remarkably specific. Identical GTP-sensitivity was observed using either purified muscle GTP or synthetic GTP formed by phosphorylation of purified GMP.

The effects mediated by other guanine nucleotides indicate that the process of Ca²⁺ release involves GTP hydrolysis. Thus, as shown in Fig. 2a, the non-hydrolysable analogue of GTP, guanosine 5'-(β,γ-imido)triphosphate (GppNHp), at 20 μM was unable to induce Ca²⁺ release. Similarly, guanosine 5'-O-(3-thio)triphosphate (GTPγS) up to 100 μM had no effect (see below). In view of this apparent requirement for terminal phosphate hydrolysis, it was perhaps surprising that 20 μM GDP induced an almost maximal release of Ca²⁺ (Fig. 2a). However, this occurred over an extended time period (several minutes) and only after a lag of ~30 s before significant Ca²⁺ release was observed. This effect is explained by conversion of GDP to GTP catalysed by nucleoside diphosphokinase activity, with the high ATP concentration serving as a phosphoryl donor (see Fig. 2b). Thus, when 1 mM ADP (effective at blocking diphosphokinase activity with an inhibition constant K_i of 26 μM; ref. 20) was added together with GDP, the action of the latter was completely abolished due to the lack of its enzymatic phosphorylation to GTP. On the other hand, ADP had no effect on GTP-mediated Ca²⁺ release, nor did it alter equilibrium Ca²⁺ accumulation in the absence of guanine nucleotides. Guanosine 5'-O-(2-thio)diphosphate (GDPβS), a GDP analogue which does not undergo phosphorylation, was also ineffective in inducing significant Ca²⁺ release.

Most importantly, GDP is not only ineffective in directly inducing Ca²⁺ release, but is in fact a highly effective and specific inhibitor of GTP-mediated Ca²⁺ release. Thus, as shown in Fig.

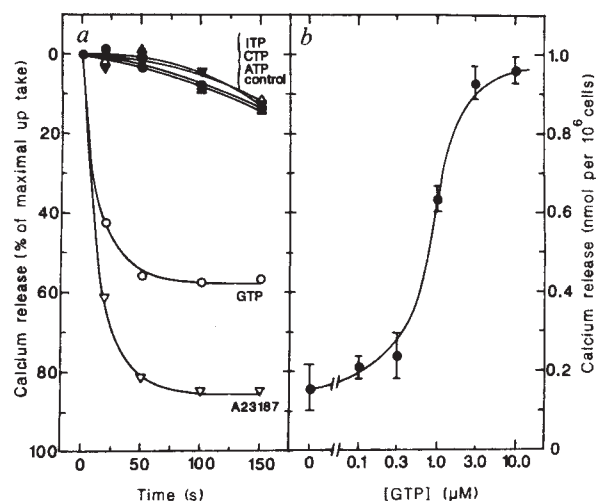
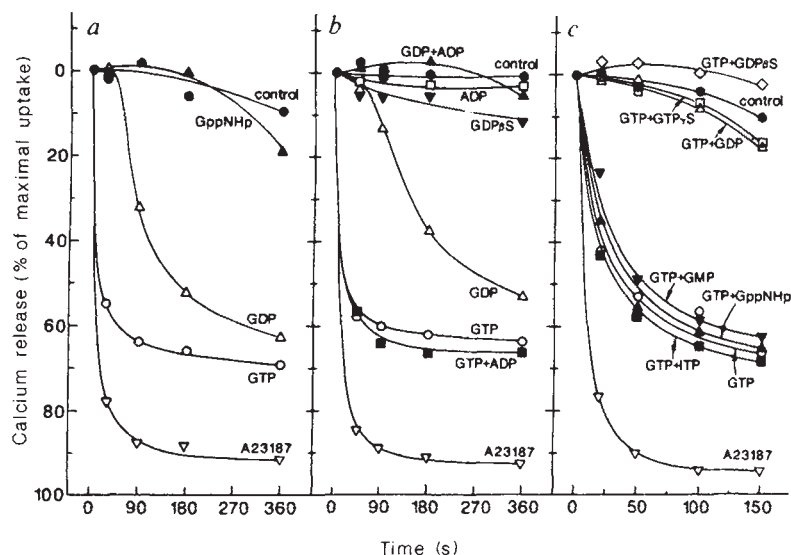


Fig. 1 Nucleoside triphosphate specificity (a) and GTP-dependence (b) of Ca²⁺ release from permeabilized N1E-115 cells. Conditions for the growth, permeabilization and uptake of Ca²⁺ for both experiments were similar to those described previously⁶. Briefly, cells cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum for 12 days, were removed from culture dishes using D₁ medium (137 mM NaCl, 5.4 mM KCl, 58 mM sucrose, 5.5 mM glucose, 0.17 mM Na₂HPO₄, 0.22 mM KH₂PO₄, pH 6.8) and resuspended in intracellular-like medium (IM; 140 mM KCl, 10 mM NaCl, 2.5 mM MgCl₂, 10 mM HEPES-KOH, pH 7.0) at 2×10^6 cells ml⁻¹. After treatment with 0.005% saponin for 10 min at 37 °C, permeabilized cells (comprising 98% of total cells) were washed three times with saponin-free IM. a, Permeabilized cells resuspended at 0.37×10^6 cells ml⁻¹ were incubated at 37 °C in gently stirred vials containing 1.5 ml of 10 μM CaCl₂ (with 80 Ci per mol ⁴⁵Ca), 1 mM ATP, 3% PEG (relative molecular mass 6,000) and 48.7 μM EGTA (to give 0.1 μM free Ca²⁺). Mitochondrial inhibitors used previously⁶ were omitted as uptake at 0.1 μM free Ca²⁺ comprises a negligible mitochondrial component. ATP-dependent uptake of Ca²⁺ within the cells proceeded for 4 min, at which time duplicate samples (200 μl each) were immediately removed either before or at the indicated times after addition of 10 μM GTP (○), 10 μM ITP (▲), 10 μM CTP (△), 10 μM ATP (▼), 5 μM A23187 (▽) or IM control (●), each in 1% of the remaining volume. Each 200-μl sample was immediately diluted into 2.5 ml of IM containing 1 mM LaCl₃ at 4 °C, and separated on glass-fibre filters (type 31, Schleicher & Schuell) which were rapidly washed twice with 2.5 ml of La³⁺-containing IM. Results are Ca²⁺ release as a percentage of maximal uptake immediately before addition (in this assay, 1.135 ± 0.074 nmol Ca²⁺ per 10⁶ cells). b, The conditions for Ca²⁺ uptake were the same as for a except that cells cultured for 11 days were present in the assay at 0.54×10^6 cells ml⁻¹, ATP-dependent Ca²⁺ accumulation proceeded for 5 min at 37 °C, and release of Ca²⁺ after addition of the indicated concentration of GTP was for 4 min in each case. Each result is the mean $\pm$ s.d. of six measurements (triplicate determinations taken from two separate vials at each GTP concentration). The mean Ca²⁺ accumulation for all vials at the end of the 5-min uptake period was 1.416 ± 0.101 nmol Ca²⁺ per 10⁶ cells.

2c, 100 μM GDP (a concentration which saturates diphosphokinase activity, and consequently is not significantly converted to GTP) or GDPβS completely blocked the action of GTP. However, at the same high competing concentrations, GppNHp, GMP and ITP were all ineffective in blocking the action of GTP, indicating the considerable structural specificity for molecules interacting with the GTP site. The blocking action of GDP further implies that GTP hydrolysis is involved in activation of Ca²⁺ release, since, if GDP is formed and remains at least transiently associated with the active site, then a high concentration of added GDP may inhibit GDP release and hence prevent further GTP association. If GTP hydrolysis is required, why does GppNHp not block the action of GTP? The answer seems to be that this particular GTP analogue may simply not be recognized by the GTP site. In contrast, GTPγS completely

Fig. 2 Effect of various guanine nucleotides on the release of Ca^{2+} from permeabilized N1E-115 cells. Experiment *a*: ●, buffer control; ○, 10 μM GTP; ▲, 20 μM GppNHP; △, 20 μM GDP; ▽, 5 μM A23187. Experiment *b*: ●, buffer control; ○, 10 μM GTP; △, 10 μM GDP; □, 1 mM ADP; ■, 10 μM GTP with 1 mM ADP; ▲, 10 μM GDP with 1 mM ADP; ▼, 10 μM GDP β S; ▽, 5 μM A23187. Experiment *c*: ●, buffer control; ○, 3 μM GTP; ▲, 3 μM GTP with 100 μM GppNHP; △, 3 μM GTP with 100 μM GDP; ▼, 3 μM GTP with 100 μM GMP; ■, 3 μM GTP with 100 μM ITP; □, 3 μM GTP with 100 μM GTP γ S; ◇, 3 μM GTP with 100 μM GDP β S; ▽, 5 μM A23187. Total Ca^{2+} accumulation in the vials immediately before the additions in experiments *a*–*c* was 2.162 ± 0.122 , 1.505 ± 0.124 and 1.316 ± 0.086 nmol Ca^{2+} per 10^6 cells, respectively.

Methods. Cells were loaded with Ca^{2+} under the conditions described in Fig. 1 legend, except that the incubations contained 9–14-day cultured cells at $0.24\text{--}0.52 \times 10^6$ cells ml^{-1} . Additions were made at the end of 4-min uptake periods, incubations being continued for the times indicated, followed by rapid addition of La^{3+} -medium and filtration, as described in Fig. 1 legend.



blocks the action of GTP (Fig. 2c), indicating that this triphosphate can indeed bind to the GTP site, its ineffectiveness substantiating the requirement for terminal phosphate hydrolysis in the activation process.

Support for the contention that GDP interacts with the same site as GTP derives from competition studies (see Fig. 3a). Thus, the effects of two concentrations of GDP (10 and 100 μM) on the GTP-dependence of Ca^{2+} release were examined in the presence of ADP (to inhibit conversion of GDP to GTP). The results show that GDP induces only a decrease in sensitivity to GTP with no change in its maximal effectiveness. Thus, Lineweaver-Burk analysis (Fig. 3a, inset) revealed a change only in the apparent K_m for GTP (~ 0.75 μM in the absence of GDP), indicating a simple competitive inhibition by GDP, with a K_i of 3.1 μM . The ability of both GTP and GDP to associate with the same site constitutes further evidence that a hydrolytic reaction occurs.

The specific inhibition by GDP provides an important means of determining the relationship between GTP-dependent Ca^{2+} release and that mediated by InsP_3 . Thus, the permeabilized N1E-115 cells demonstrate a rapid Ca^{2+} release response to InsP_3 . As shown in Fig. 3b, the release induced by 10 μM InsP_3 remained unaltered even in the presence of 0.5 mM GDP (Fig. 3b), indicating an important distinction between the two release mechanisms. An even more significant difference is reflected by the temperature-dependence of GTP-induced Ca^{2+} release (data not shown) whereby the rate of GTP-mediated Ca^{2+} release, normally conducted at 37 $^\circ\text{C}$, was reduced fourfold at 19 $^\circ\text{C}$, and to virtually zero at 2 $^\circ\text{C}$; this contrasts with the recently described temperature-insensitivity of InsP_3 -induced Ca^{2+} release²¹, and suggests strongly that an enzyme mediates the effects of guanine nucleotides on Ca^{2+} release.

From Figs 1–3 it is clear that GTP never mediates 100% release of ionophore-releasable Ca^{2+} . There are several possible explanations for this effect, including: (1) reduction in the availability of GTP; (2) a change in the releasability of internal Ca^{2+} after GTP addition; (3) refractoriness of the release mechanism; and (4) the existence of discrete subcompartments of ER. The study described in Fig. 4 resolves certain of these possibilities. Thus, the re-addition of GTP after maximal GTP-mediated Ca^{2+} release resulted in no further release of Ca^{2+} from the cells, whereas application of ionophore at this time released all remaining releasable Ca^{2+} . These results indicate first, that lack of GTP is not limiting, and second, that the internal Ca^{2+} remains fully releasable. That the release mechanism does in fact remain activated is implied by re-addition of

ATP (to compensate for any ATP loss during the experiment) after GTP-mediated release, whereupon no re-uptake of Ca^{2+} was observed. This view is extended by the important observation that addition of GDP after GTP-mediated release reversed the release process and resulted in at least partial re-accumulation of Ca^{2+} (Fig. 4, inset). This result indicates that with GDP competing for the regulatory site and preventing either GDP

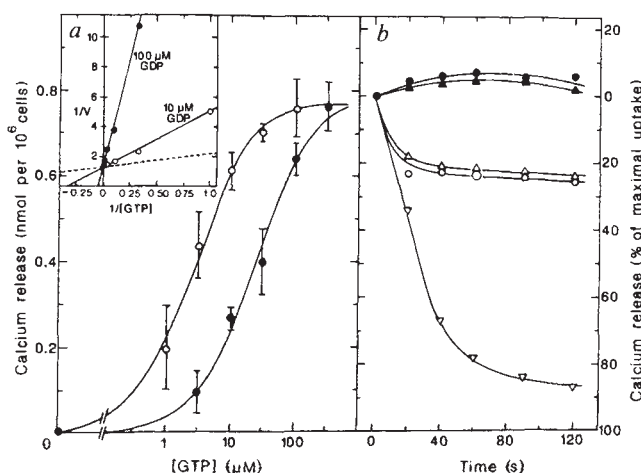


Fig. 3 Effect of GDP on the concentration-dependence of GTP-mediated Ca^{2+} release (*a*) and on InsP_3 -mediated Ca^{2+} release (*b*) from permeabilized N1E-115 cells. Experimental conditions were as described in Fig. 1 legend. *a*, Cells cultured for 8 days were present at 0.21×10^6 cells ml^{-1} , and Ca^{2+} uptake proceeded for 5 min, followed by a 2-min period of Ca^{2+} release in the presence of 1 mM ADP and the indicated concentration of GTP, together with either 10 μM (○) or 100 μM (●) GDP. GTP-dependent Ca^{2+} release was the difference between Ca^{2+} release in the presence and absence of GTP, each result being the mean $\pm$ s.d. of triplicate determinations. Total Ca^{2+} accumulation in each of the vials immediately before guanine nucleotide addition was 1.544 ± 0.135 nmol Ca^{2+} per 10^6 cells. Inset, Lineweaver-Burk analysis of the data, together with data on GTP-dependence obtained in the absence of GDP (dotted line), for comparison. *b*, 12-day cells were present at 0.23×10^6 cells ml^{-1} , and Ca^{2+} uptake proceeded for 5 min, followed by Ca^{2+} release for the indicated times in the presence of: ●, buffer control; ○, 10 μM InsP_3 ; ▲, 0.5 mM GDP; △, 10 μM InsP_3 with 0.5 mM GDP; ▽, 5 μM A23187. Total Ca^{2+} accumulation in each vial before additions was 1.658 ± 0.060 nmol Ca^{2+} per 10^6 cells.

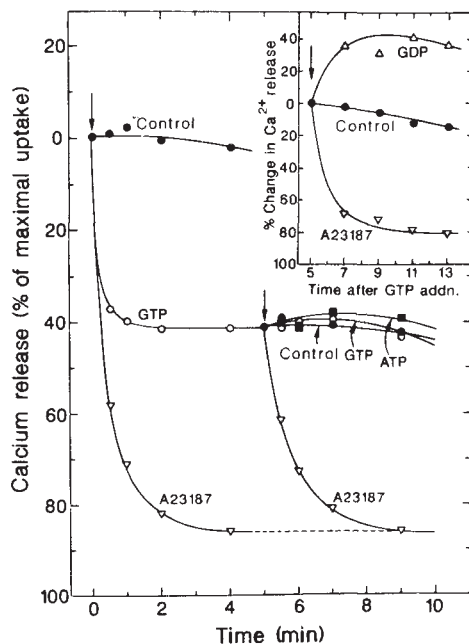


Fig. 4 Effects of GTP, GDP, ATP and A23187 on Ca^{2+} release from permeabilized cells after maximal GTP-mediated Ca^{2+} release. Cells cultured for 10 days were present at 0.66×10^6 cells ml^{-1} , with initial Ca^{2+} accumulation proceeding for 5 min. At this time ($t=0$) Ca^{2+} release was measured after addition of either control buffer (●), 10 μM GTP (○) or 5 μM A23187 (▽), for the times indicated. Additional vials which had been subjected to release with 10 μM GTP for exactly 5 min, received further additions at this time of either control buffer (●), 10 μM GTP (○), 1 mM ATP (■) or 5 μM A23187 (▽), release proceeding for the times indicated. Total Ca^{2+} accumulation in each of the vials before the first additions was 1.170 ± 0.182 nmol Ca^{2+} per 10^6 cells. The inset shows an identical experiment in which maximal release of accumulated Ca^{2+} was effected with 10 μM GTP for 5 min, followed by addition of either control buffer (●), 500 μM GDP (△) or 5 μM A23187 (▽). In this experiment cells were present at 0.93×10^6 cells ml^{-1} , and Ca^{2+} uptake before GTP addition was 0.822 ± 0.048 nmol Ca^{2+} per 10^6 cells.

dissociation and/or GTP reassociation, the release mechanism is returned to an inactive state, allowing Ca^{2+} to be retained within the ER.

Thus, the fact that GTP consistently induces only partial release of Ca^{2+} with respect to total releasable Ca^{2+} probably reflects heterogeneity of the Ca^{2+} -sequestering organelles, an effect similar to that observed for InsP_3 -mediated Ca^{2+} release in both permeabilized N1E-115 cells and other cells^{1,2,5,8-10}. The profound effectiveness of GTP alone in inducing Ca^{2+} release from permeabilized cells contrasts with the apparent lack of effect of the nucleotide on liver microsomes as reported by Dawson¹⁶, except in the presence of InsP_3 . This difference is apparently not accounted for by the types of preparation used, as GTP alone also induces substantial Ca^{2+} release from N1E-115 cell-derived microsomes, as we recently described²². Although differences in the levels of endogenous InsP_3 could account for such a divergence, the effectiveness of InsP_3 (half-maximal at 0.5 μM) in inducing Ca^{2+} release from permeabilized N1E-115 cells in the absence of GTP militates against this conclusion. Dawson¹⁶ also showed that in order for GTP to have an effect on InsP_3 -mediated Ca^{2+} release from liver microsomes polyethylene glycol (PEG) must be present. With permeabilized N1E-115 cells, GTP directly activates Ca^{2+} release without PEG, however 3% PEG was included in our experiments as its addition enhanced the extent of GTP-mediated Ca^{2+} release. Interestingly, GTP-mediated Ca^{2+} release from N1E-115 cell microsomes is completely dependent on PEG²², suggesting that the release mechanism may involve the interac-

tion of different membrane and/or protein components of ER, which may become dissociated or damaged during homogenization of the cells. Since microtubule assembly is both promoted by PEG²³ and dependent on GTP hydrolysis²⁴, one hypothesis was that polymerization or attachment of microtubules might be involved in the Ca^{2+} -release mechanism. However, when the microtubule assembly inhibitor colchicine was added to the cell growth medium and/or included in the release assay, it neither blocked nor prevented the GTP-mediated Ca^{2+} release.

These results provide direct evidence for a specific guanine nucleotide-regulated Ca^{2+} release mechanism in ER which requires GTP, is competitively inhibited by GDP, and probably entails GTP hydrolysis. GTP-induced Ca^{2+} release is sufficiently large and rapid to indicate the operation of a channel across the ER membrane. However, the activation of such a channel appears to be controlled by a temperature-sensitive enzymatic reaction involving GTP hydrolysis. Although at present it is unclear whether a simple GTPase activity regulates activation or whether phosphorylation of a regulatory protein occurs via a GTP-dependent kinase reaction, the effect of GDP on reversing the system suggests the former mechanism. Such a mechanism resembles guanine nucleotide regulation of adenylate cyclase activation²⁵, with GDP stabilizing a non-activated state, GTP effecting activation (with submicromolar sensitivity), and inter-conversion between the two states being mediated by a GTPase activity; however, only in the present case is nucleotide hydrolysis required for activation. This mechanism also indicates that the ratio of GTP to GDP may be a determining factor in the opening or closing of Ca^{2+} channels within ER, the operation of such channels thus being directly related to the energy charge of the cell. Other regulatory or modifying factors (perhaps controlling the GTPase activity) are likely to be involved in the physiological control of the GTP-mediated release mechanism, particularly as implied by the effectiveness of PEG. Although the GTP-mediated release mechanism has been studied under conditions in which it is functionally distinct from InsP_3 -mediated release, at least with respect to both GDP-induced inhibition and temperature sensitivity, further experiments are needed to assess the intriguing possibility that the two mechanisms are interrelated.

This work was supported by NIH grant NS19304 by NSF grant DCB-8510225, and by the American Heart Association, Maryland Affiliate. We thank Dr Robin Irvine for supply of InsP_3 , and Drs Martin Rodbell and Dermot Cooper for helpful discussions and advice.

Received 16 December 1985; accepted 5 February 1986.

- Berridge, M. J. & Irvine, R. F. *Nature* **312**, 315-321 (1984).
- Gill, D. L. *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* **19**, 195-212 (1985).
- Wakasugi, H. *et al. J. Membrane Biol.* **65**, 205-220 (1982).
- Burgess, G. M., McKinney, J. S., Fabiato, A., Leslie, B. A. & Putney, J. W. *J. biol. Chem.* **258**, 15336-15345 (1983).
- Gershengorn, M. C., Geras, E., Purrello, V. S. & Rebecchi, M. J. *J. biol. Chem.* **259**, 10675-10681 (1984).
- Gill, D. L. & Chueh, S. H. *J. biol. Chem.* **260**, 9289-9297 (1985).
- Streb, H., Irvine, R. F., Berridge, M. J. & Schulz, I. *Nature* **306**, 67-69 (1983).
- Burgess, G. M. *et al. Nature* **309**, 63-66 (1984).
- Joseph, S. K., Thomas, A. P., Williams, R. J., Irvine, R. F. & Williamson, J. R. *J. biol. Chem.* **259**, 3077-3081 (1984).
- Hirata, M., Suematsu, E., Hashimoto, T., Hamachi, T. & Koga, T. *Biochem. J.* **223**, 229-236 (1984).
- Joseph, S. K., Williams, R. J., Corkey, B. E., Matschinsky, F. M. & Williamson, J. R. *J. biol. Chem.* **259**, 12952-12955 (1984).
- Prentki, M., Wollheim, C. B. & Lew, P. D. *J. biol. Chem.* **259**, 13777-13782 (1984).
- Prentki, M. *et al. Nature* **309**, 562-564 (1984).
- Streb, H., Bayerdörffer, E., Haase, W., Irvine, R. F. & Schulz, I. *J. Membrane Biol.* **81**, 241-253 (1984).
- O'Rourke, F. A., Halenda, S. P., Zavoico, G. B. & Feinstein, M. B. *J. biol. Chem.* **260**, 956-962 (1985).
- Dawson, A. P. *FEBS Lett.* **185**, 147-150 (1985).
- Gill, D. L., Grollman, E. F. & Kohn, L. D. *J. biol. Chem.* **256**, 184-192 (1981).
- Gill, D. L. *J. biol. Chem.* **257**, 10986-10990 (1982).
- Gill, D. L., Chueh, S. H. & Whitlow, C. L. *J. biol. Chem.* **259**, 10807-10813 (1984).
- Kimura, N. & Shimada, N. *J. biol. Chem.* **258**, 2278-2283 (1983).
- Smith, J. B., Smith, L. & Higgins, B. L. *J. biol. Chem.* **260**, 14413-14416 (1985).
- Ueda, T., Chueh, S. H., Noel, M. W. & Gill, D. L. *J. biol. Chem.* **261**, 3184-3192 (1986).
- Lee, J. C. & Lee, L. Y. *Biochemistry* **18**, 5518-5526 (1979).
- Timasheff, S. N. & Grisham, L. M. *A. Rev. Biochem.* **49**, 565-591 (1980).
- Rodbell, M. *Nature* **284**, 17-22 (1980).

New evidence for the dendrochronological dating of Netherlandish paintings

THE recent paper by Baillie *et al.*¹ resolves a number of long-standing problems in art-historical dendrochronology. One of the first uses of dendrochronology in the history of art dealt with paintings of the Dutch painter Philips Wouwerman who spent all his life in Amsterdam^{2,3}. The panels were successfully dated using the South German oak tree-ring chronology⁴, one of only three European long-term oak chronologies in existence at the time. Since the sixteenth/seventeenth century section of the reference series contained timber derived mainly from Hesse in central Germany, the panels were assumed to be from trees cut in the Hessian hills and transported to the Netherlands. Many of these panels had some sapwood remaining, so the felling dates of the oak trees were estimated using an allowance for sapwood of 20 ± 5 years. This figure is of the same order of magnitude as one derived from some 500 oaks from along both sides of the river Rhine⁵.

The problem with art-historical dendrochronology arose when analysis of oak panels used by Rembrandt⁶, Rubens⁷ and other painters between the fourteenth and the middle of the seventeenth centuries revealed a new tree-ring pattern which did not cross-match with any of the existing oak chronologies. Since most of the tree-ring series of these panels matched each other extremely well, a floating chronology covering 530 years was established, and this new tree-ring pattern was initially thought to be characteristic for the Netherlands. This assumption was supported by the fact that the panels of English paintings showed an identical pattern⁸, and a growing region along both sides of the Channel was envisaged. It seemed unlikely that the timbers had been imported into the Netherlands since this would mean that trade connections from the fourteenth to the seventeenth century always brought timbers from the same source. But it was striking that no painting from later than around 1650 could be found with the new tree-ring pattern.

In an attempt to tie down this alternative chronology, a complete Netherlands tree-ring chronology was constructed back to AD 1036⁹. However, the alternative tree-ring pattern still failed to crossdate with the Netherlands chronology. It became apparent that two tree-ring chronologies existed for the Netherlands: Type I, relevant to building timbers; and Type II from panel paintings. The position in time of the Type II chronology was assigned on the basis of some ring patterns which appeared to show similarities with both Types¹⁰.

In the meantime a number of new long-term oak chronologies have come into

existence, so that from South Sweden through Central to Western Europe a dense grid of reference material is now available¹¹. In the light of this new material and with a better understanding of the degree of similarity which can be expected between chronologies from distant localities, the idea that the Type II tree-ring pattern derived from the Netherlands became increasingly untenable.

Within the natural distribution of oak in Europe (Fig. 1) the eastern Baltic coast represents the most likely export source for oak timbers to the Netherlands.

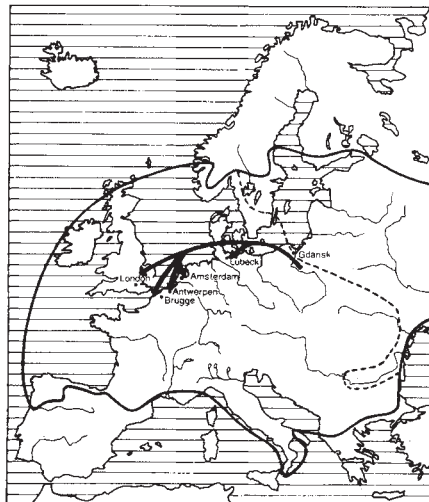


Fig. 1 The areas of the natural distribution of oak. Distribution of *Quercus robur* L. (European oak) is shown as a heavy line, and *Quercus petraea* Liebl. (sessile oak) as an interrupted line. European oak goes further north-east than sessile oak. The source of oak timbers of type II tree-ring pattern and the places of their utilization as panels are indicated (arrows).

According to historical and archival evidence¹²⁻¹⁴ the Vistula river (Weichsel) and its tributaries made the large Polish forests accessible. But despite this endem and the independent investigation of the English Type A art-historical chronologies (which matched with Netherlands Type II) by Baillie *et al.*, which suggested that the timber had derived from Eastern Europe, possibly Poland or Lithuania, a definitive dendrochronological proof was still missing.

In an attempt to finally resolve this question, we have undertaken a study of building timbers from churches and other historical buildings, from art-historical objects and archaeological excavations in North Poland around Gdansk. A chronology has now been constructed from 1985 back to AD 1000 using some 300 tree-ring series. This chronology matches the Type II chronology with a high 't' value of 6.5 and a percentage of parallel variation of 60%. The Type II chronology now covers the time span from 1115 to 1643 and thus has to be shifted by six years towards the present from our original tentative placement.

With the origin of the Type II timbers resolved it is interesting to trace their occurrence. They are present in Antwerp⁷, Amsterdam⁶, Brussels¹⁵, Cologne¹⁶ and Lübeck (Eckstein, unpublished data), confirming the trade connections indicated in Fig. 1. Timbers with the Type II ring pattern have only once been found as building timbers in Amsterdam, which supports the view that most of the Baltic oak was exported in the form of planks and boards. An explanation of why Type II timbers are never found after 1650 can be seen in the consequences of the Thirty

Table 1 Ten dendrochronologically dated panels of Rembrandt paintings

Paintings	Time signature	Number of measurable:		Dendrochronological felling range of the oak trees:	
		tree rings altogether	sapwood rings	before correction with a sapwood allowance of 20 ± 5 yr	after correction with a sapwood allowance of 13...15...19yr
Br. 486 Anna accused by Tobit of stealing the kid	1626	254	5	1622 ± 5	1621...1627
Br. 489 Samson betrayed by Delilah	1628	277	6	1623 ± 5	1622...1628
Br. 2 Self-portrait	1629	132	5	1630 ± 5	1629...1635
Br. 165 Aelbert Cuyper	1632	168	9	1630 ± 5	1629...1635
BR. 152 Old man with a gold chain	1632	132	4	1614 ± 5	1613...1620
Br. 157 A Self-portrait	1632	133	7	1632 ± 5	1631...1637
Br. 159 Portrait of a man	1632	185	4	1633 ± 5	1632...1638
Br. 336 Cornelia Pronck, wife of Aelbert Cuyper	1633	184	3	1632 ± 5	1631...1637
Br. 206 A man in oriental costume	1635	309	11	1626 ± 5	1625...1632
Br. 211 A man in Polish costume	1637	188	7	1634 ± 5	1633...1639

Each dated panel has a time signature; the former and the new range for the felling year of the oak trees are compared taking into account the respective sapwood allowances.

Years War which cut the trade routes across the Baltic Sea. Finally the Second Swedish/Polish War from 1655 to 1660 caused the total breakdown of the Weichsel trade¹³.

With the detection of the provenance for the oak panels new evidence for the sapwood allowance arises. A preliminary analysis of the number of sapwood rings from 180 oaks, from North Poland, results in a median value of 15 with 50% of all values lying between 13 and 19, the lower and upper extremes are 9 and 36, so far. The indication of a west-east gradient of decreasing sapwood rings¹ is thus supported. On the basis of the new placement-in-time and the new sapwood allowance the dating of ten Rembrandt paintings are summarized in Table 1. All ten panels have some sapwood remained and the paintings contain an authentic time signature. Since through the correction a net-shift of only one year resulted, there are no serious art-historical consequences for the respective paintings. All these considerations also apply to the English art-historical objects of the same tree-ring pattern¹, but not to other art-historical exercises. Thus dates established for oak panels, sculptures¹⁷ and furniture¹⁸ with ring patterns of genuine Dutch³, French¹⁹ or West German¹⁶ type are unaffected as are dates for beech panels bearing paintings by artists such as Lucas Cranach²⁰, and stringed instruments of spruce²¹.

We thank the Polish Academy of Science (PAN) in Warszawa and the German Academic Exchange Service (DAAD) in Bonn for their generous financial support. This work contains parts of a doctoral dissertation of T.W.

D. ECKSTEIN*
T. WAZNY†
J. BAUCH*
P. KLEIN*

* Institute for Wood Biology,
University of Hamburg, Leuschnerstr. 91
D-2050 Hamburg 80, FRG
† Academy of Fine Arts,
Conservation Faculty,
Wybrzeże Kościuszkowskie 37,
00-379 Warszawa, Poland

1. Baillie, M. G. L., Hillam, J., Briffa, K. R. & Brown, D. M. *Nature* **315**, 317-319 (1985).
2. Bauch, J. *Kunstchronik* **21**, 144-145 (1968).
3. Bauch, J. & Eckstein, D. *Stud. Conserv.* **15**, 45-50 (1970).
4. Huber, B. & Gierz, V. *Conf. Rep. Austrian Acad. Sci.* **178**, 32-42 (1969).
5. Hollstein, E. *Mitteleuropäische Eichenchronologie* (Zabern, Mainz, 1980).
6. Bauch, J., Eckstein, D. & Meier-Siem, M. *Ned. Kunsthistorisch Jb.* **23**, 485-496 (1972).
7. Bauch, J., Eckstein, D. & Brauner, G. *Jb. Berliner Museen* **20**, 209-221 (1978).
8. Fletcher, J. M. *Burlington Mag.* **116**, 250-258 (1974).
9. Eckstein, D., Brongers, J. A. & Bauch, J. *Tree-Ring Bull.* **35**, 1-13 (1975).
10. Bauch, J. *Br. archaeol. Rep. (Int. Ser.)* **51**, 133-137, 307-314 (1978).
11. Eckstein, D. & Wrobel, S. *Dendrochronologia* **1**, 9-20 (1985).
12. Rath, R. E. *Der Weichselhandel in 16. Jh.* (Marburg, 1927).
13. Krannhals, D. *Danzig und der Weichselhandel in seiner Blütezeit im 16. und 17. Jh.* (Leipzig, 1942).

14. Bitvinkas, T. T., Brukštus, V. & Zulkus, V. V. *Ist. Botan. AN Litov. SSR*, 69-73 (1984).
15. Klein, P. *5th Int. Seminar Appl. of Science in Examination of Works of Art*, Boston (1983).
16. Eckstein, D. & Bauch, J. *Mitt. Deutschen Dendrol. Ges.* **67**, 247-256 (1974).
17. Becker, B. *Jahrbuch der staatlichen Kunstsammlungen in Baden-Württemberg* **16**, 28-32 (1974).
18. Bauch, J. & Eckstein, D. *Niedersächsische Denkmalpflege* **9**, 116-124 (1978).
19. Klein, P. *ICOM 7th Triennial Meeting*, Copenhagen (1984).
20. Klein, P. & Bauch, J. *Holzforschung* **37**, 1, 35-39 (1983).
21. Klein, P., Mehringer, E. & Bauch, J. *ICOM, 7th Triennial Meeting*, Copenhagen (1984).

Dating of art-historical artefacts

FROM statistical studies, Baillie *et al.*¹ propose the re-dating, which we accept, of oak tree-ring chronologies² referred to by us as Type A, and make suggestions, to which we here add information, concerning the provenance of the timber and the relevance of absent sapwood to the dating of artefacts of art-historical interest in northern Europe^{2,3}.

The first suggestion is that oak timber exported westward from east Prussia and Poland to Flanders and England was used for the panels of many late mediaeval paintings now in Flanders and England. The Type A chronologies largely derived from the timbers cover the years AD 1250-1550, which coincides with the activity of Hanse merchants^{4,5}. We find further support for the Baltic provenance in that the species of oak of many such panels have affinity to pedunculate oak, which is more prevalent than sessile oak in eastern Europe⁶. This evidence derives from wood structure analysis on the exposed edges of the panels with rings sufficiently wide (>2 mm) to permit classification by the method of Huber *et al.*⁷. Introgression of the two species being common in north-west Europe^{8,9}, it is not feasible to identify the species for many samples, but finding an affinity to one or the other is possible. Our finding that Type A panels have affinity to pedunculate oak is relevant to the poor match noted between Type A and Type H (sessile oak) chronologies. This is consistent with the finding of Cousens⁹ that woodlands dominated by pedunculate oak exhibit greater variability than those with sessile oak.

The other points of Baillie *et al.*¹ concern first, movement of our Type A chronologies forward by 4 years, and second, the relatively low number of sapwood years on Baltic oak. We suggested the former at one time¹⁰ but for various reasons replaced it with chronologies in other positions. However, we substantiate the dating of Type A chronologies now proposed by Baillie *et al.* Their second suggestion was deduced from *Quercus robur* on Finnish sites for oak 120 yr old and confirms what has already been reported for sapwood by Bräthen¹¹ for west Sweden. Bräthen examined mediaeval oak

from excavations at Lödse on the Gotha River and for 69 trees found means of 16 yr (range 13-21 yr) for trees 100-200 yr old, and 20 yr (range 14-23 yr) for older trees. In contrast to the research on sessile oak referred to by Baillie, and on modern oak in Ireland and north-west England, our experience for pedunculate oak in southern England in Bagley Wood¹² and for mediaeval⁸ and Roman¹³ times indicates that trees of fast growth—that is, with wide rings—have relatively few sapwood rings.

The interesting suggestions raised by Baillie *et al.* have added to our knowledge, and the long-standing problem of dating Type A chronologies of slow growth has been resolved. The suggestions do not significantly affect our results for Tudor and other paintings on panel, for which our aim has been to derive the probable date to within 15-20 yr.

J. M. FLETCHER

20 Tullis Close,
Sutton Courtenay,
Abingdon, Oxfordshire OX14 4BD, UK

1. Baillie, M. G. L., Hillam, J., Briffa, K. R. & Brown, D. M. *Nature* **315**, 317-319 (1985).
2. Fletcher, J. M. & Tapper, M. C. *Medieval Archaeol.* **28**, 112-132 (1984).
3. Klein, P. in *Dendrochronology and Archaeology in Europe*, Hamburg, 209-222 (1983).
4. Dollinger, P. *The German Hanse* (Macmillan, London, 1970).
5. Bitvinkas, T. (ed.) *Climatic Change in Time and Space and Annual Tree Rings* 69-74 (Kaunas, Lithuanian SSR, USSR, 1984).
6. Rubner, K. & Reinhold, F. *Das Natürliche Waldbild Europas* (Publisher Paul Parey, Hamburg, 1953).
7. Huber, B., Hoidheide, W. & Raack, K. *Holz als Roh- und Werkstoff* **4**, 373-380 (1941) 194-221.
8. Longman, K. A. & Coutts, M. P. *The British Oak* (Botanical Society of the British Isles, Classey, Faringdon, 1974).
9. Cousens, J. E. *Watsonia* **6**, 161-176 (1965).
10. Fletcher, J. M. *Nature* **254**, 506-507 (1975).
11. Bräthen, A. *RAA Vol. 1*, 8-124 (National Antiquities & National Historical Museums, Stockholm, 1982); *PACT* **7**, Pt. 1, 27-35 (Second Nordic Conference on Scientific Methods in Archaeology, Copenhagen, 1982).
12. Fletcher, J. M. in *The British Oak*, 80-97 (Botanical Society of the British Isles, Classey, Faringdon, 1974).
13. Fletcher, J. M. *Trans. Lond. Middx. archaeol.* **33**, 79-84 (1982).

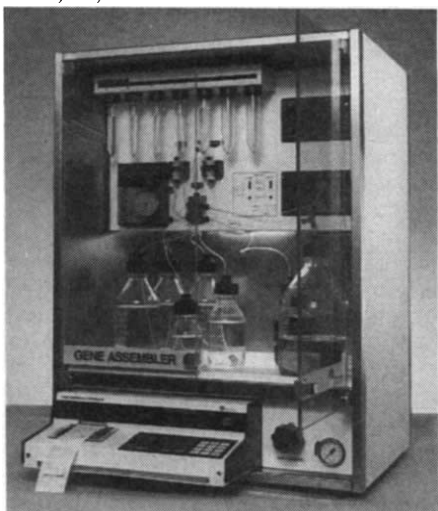
Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 500 words and the briefest of replies, to the effect that a point is taken, should be considered.

Familiar sights in fresh relief

New DNA probes hit the mark, pH sensors go micro, and an advanced focused-ion-beam column reveals a startling landscape in even the most ordinary of objects.

Pharmacia has put together a **DNA synthesizer** attuned to tight budgets. The Gene Assembler system includes micro-processor control, a printer, two reaction columns and an absorbance monitor at an introductory price of \$16,499 (US) (Reader Service No. 100). The β -cyanoethyl phosphoramidite reaction effects synthesis with greater than 98 per cent accuracy, producing oligonucleotides longer than 140 bases. Pharmacia managed to keep the system's expense down by incorporating their own chromatographic components and keeping wiring and plumbing simple. They have also cut corners on the base addition cycle time, which is slightly less than 10 minutes, and their software does not allow for direct sequence coding in A, G, T and C. The two-column as-



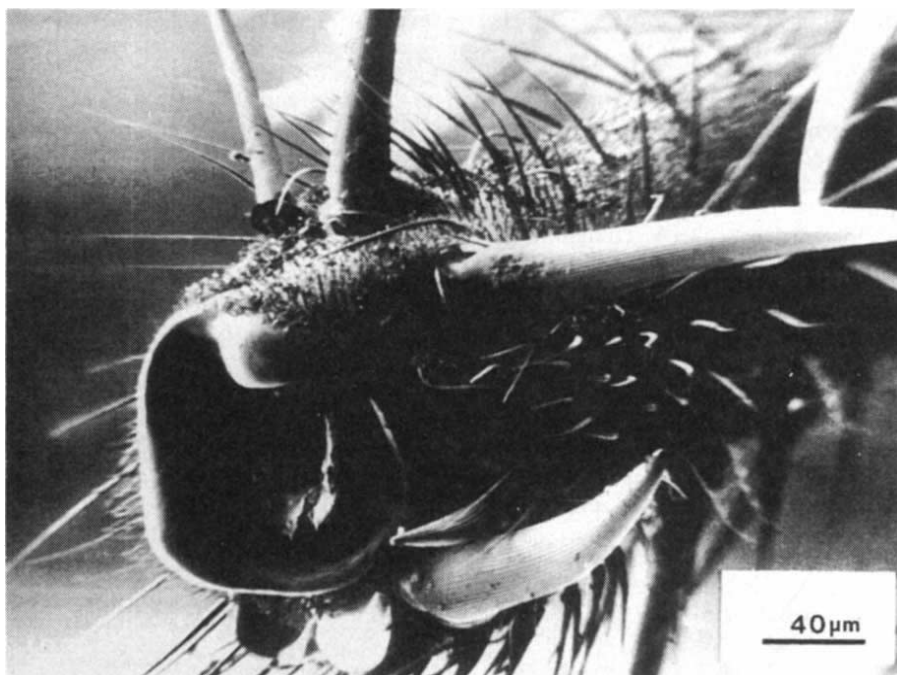
Pharmacia's DNA synthesizer monitors coupling efficiency

sembly, however, is a useful extra that some more expensive systems do not feature.

An **automated synthesizer** from Systec speeds up base additions to as little as 5 minutes while maintaining high coupling efficiencies and reagent consumption at a



Systec's new gene machine



It looks like a still from a monster movie, but this image, captured by IBT Dubilier's liquid metal focused-ion-beam column, is just a very close look at a horsefly's foot. The FIB-50 is the first commercially available column of its kind to achieve a resolution below 50 nanometres (Reader Service No. 102). The above micrograph demonstrates the instrument's performance in scanning ion microscopy. Samples, including insulators, require no preparation beyond mounting.

The FIB-50 uses gallium, which melts at

37°C, as its liquid metal source, and a two-lens focusing system that incorporates beam steer and stigmator electrodes. IBT's ambitions for the \$400,000 column go beyond its arresting imagery to the semiconductor industry, where their precise ion beam may be able to repair the masks used in chip manufacture. With present technology, expensive circuitry templates are discarded when worn. Ion beam mask repair could save the silicon set plenty of time and wasted energy, reaping rich rewards for its British originators.

cost of less than \$1.00 per cycle (Reader Service No. 101). Systec's assembler works with any current phosphate or phosphite methods, and any scale from 1/2 to 10 μ M. Menu-driven software and a colour CRT terminal make the Microsyn-1500 a pleasure to use, but not without a price: Systec's introductory offer for the system is \$28,600 (US). Oligonucleotides well in excess of 100-mers can be constructed and the controlled glass pore columns are reusable. Systec's assembly does not include a monitoring system, however, that would stop the synthesis if an incomplete coupling took place.

Bits and pieces

Supercoiled **nucleic acid markers** alleviate the need to digest DNA for sizing. Gibco BRL has increased its range of markers to include supercoiled DNA and RNA 'ladders' in broad size ranges (Reader Service

No. 103). Eleven supercoiled plasmids from 2 to 16 kb long comprise the DNA marker package, and the RNA ladders will size RNA between 0.3 and 9.5 kb. Gibco also sells a single 7.5 kb RNA marker for use in gauging the efficiency of reverse transcription copying. The markers are sold in 25 to 75 μ g quantities.

An improvement on nick translation for creating **DNA labelling probes** is being



Polymeraid makes DNA probes straight from gel fragments

promoted by P & S Biochemicals Ltd. The Polymeraid system, at \$135 (US) per kit,

generates single-stranded probes that incorporate more than 1×10^9 c.p.m. per μg from any size restriction fragment (Reader Service No. 104). Polymeraid uses fragments straight from low melting point agarose gels, eliminating the need for further purification techniques. Each kit contains reagents, buffer and nucleotides for 25 reactions.

Clontech has added several new **cDNA and genomic libraries** to their stock (Reader Service No. 105). The *Saccharomyces cerevisiae* X2180 genome is now available in lambda gt11 for \$360 (US), as is mouse spleen cDNA. Human adrenal and hepatoma cDNA in the gt11 vector can also be purchased for \$450 (US). Clontech supplies human lymphocyte cDNA in gt10 for the same price. The company has many more genomic libraries and rat, bovine and human cDNA pending release on the market.

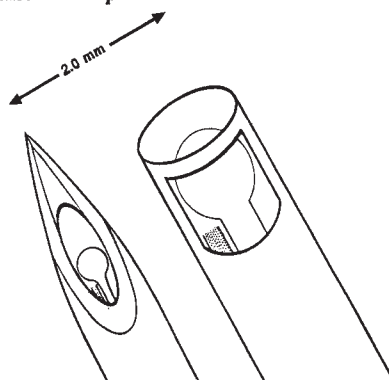
CompuGene's **DNA sequence gel reader** uses a 'talk back' feature to help prevent mistakes in sequence reading (Reader Service No. 106). With a pen to encode the base order, the analyser never has to lift his or her eyes from the gel because CompuGene's machine actually says which base it has recorded. This system hastens scanning considerably, so that 300 nucleotides can be logged in less than 5 minutes. Results from two separate readings can be compared for inconsistencies, and a final verified sequence can be stored in 15 minutes. The gel reader sells for \$4,950 (US) with a reading stand, pen,

digitizer, software and hook-up cables.

In situ hybridization with fresh or frozen cells or tissue can be accomplished through autoradiography and Oncor's hybridization kit (Reader Service No. 107). Oncor's kits will **label DNA and RNA targets** with no DNA extraction involved. Formalin-fixed, paraffin-embedded samples will not take to Oncor's method, but almost any other fresh or frozen specimen will. A complete starter kit, including reagents, protocol and equipment, costs £385 (UK) and will service 20 slide hybridizations. Stratch Scientific distributes Oncor's kit in the UK.

Acid and alkaline

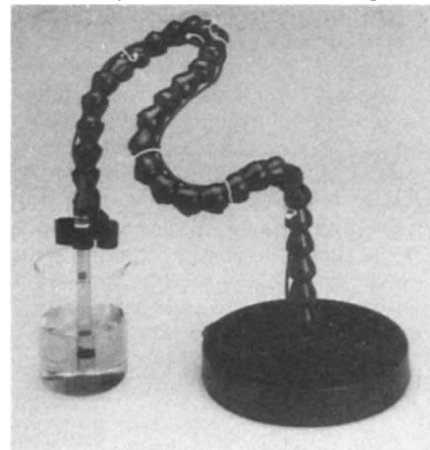
World Precision Instruments markets hand-made **pH microelectrodes** with bulbs



Big advances for small electrodes only 300 μm across (Reader Service No. 108). The 'home-brewed' glass WPI uses has one-fifth the resistance of the glass

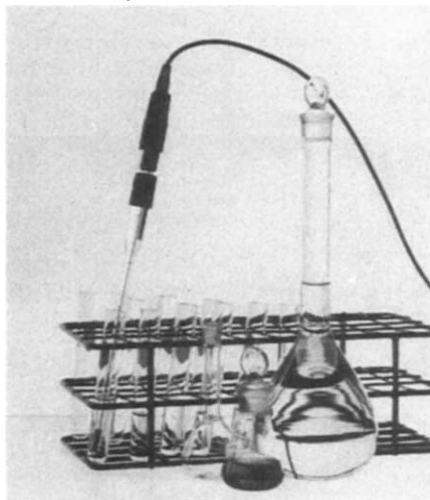
typically used in these applications, eliminating noise and improving the accuracy of microscopic measurements. The sensors are available in 20-gauge hypodermic needles 0.9 mm in diameter, or blunt-ended models with a diameter of 1.25 mm and a stem length of 100 mm. Both models cost \$295 (US) and have a shelf life of approximately one year.

The Cobra is a flexible yet very stable **holder for pH electrodes** and other probes

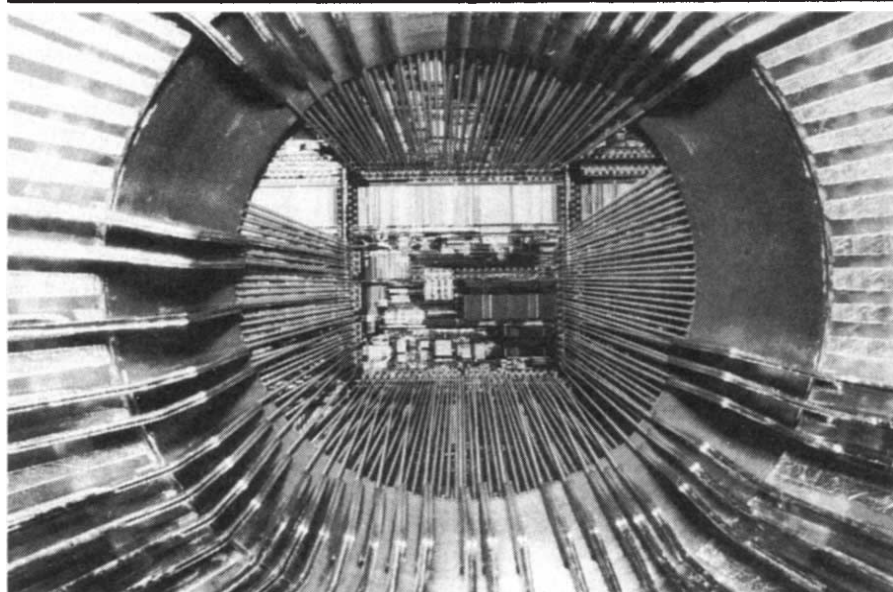


The Cobra gets a grip on pH electrodes (Reader Service No. 110). Its weighted base secures a 24-inch arm that can even bend around corners. Whatman LabSales sells extra vertebrae for the \$79 (US) Cobra, so that its length can be adjusted. The instrument's design makes traditional electrode holders seem awkward, and may save the occasional solution that takes a spill when a holder decides to go its own way.

Beckman Instruments has a **combination electrode** with a Calomel reference half-cell, small enough to fit in test tubes (Reader Service No. 111). The Futura II features a fill solution that will not react with biological and metallic samples, and comes with a one-year warranty, for \$100 (US). Its size makes it compatible with almost any glassware used by biologists.



Beckman's electrode won't alter bio-activity



Within this 32-bit chip lies the circuitry know-how for IBM's system/370, the most widely used computing instructions for their larger and faster mainframes (Reader Service No. 109). Earlier this year IBM researchers put 370 instructions directly into the microprocessor's read-only mem-

ory (ROM), and tested its operations with the 171 probes radiating from the chip's edges in the above photograph. The miniaturized 'mainframe on a chip' may enable desk-top computers to run off the same software that's the driving force behind IBM's large-scale computer success.

The 1986 pH and Conductivity Measurement Handbook and Encyclopedia from Omega lists hundreds of products in over 100 full-colour pages (Reader Service No. 112).



Omega gives a pH rundown

The catalogue gives prices, specifications, and technical data in eight sections covering everything from pH electrodes to data acquisition systems. Omega also offers catalogues on temperature and measurement, pressure and strain, and flow and level.

Databasics

CAS ONLINE, the database of the American Chemical Society, will get a 2-million-abstract boost by mid-1986. Chemical Abstracts Service has made plans to re-key the abstracts published between 1967 and mid-1975, which were never rendered in computer-readable form (Reader Service No. 113). CAS ONLINE at present covers 12,000 journals and 6.5 million papers. The new abstracts will be available at no additional cost. CAS charges an initial subscription fee of \$50 (US), with supplementary charges for access time and search answers. The service includes access to their registry file of 7.6 million chemical substances, which can be searched by name or structure. CAS expects to have a more detailed patent file and a reaction file in place sometime during 1987.

For reference searching and citation sequencing, Autoscribe Ltd has a program that can store author, year, title, source, location and key words in any specified bibliographic format (Reader Service No. 114). The Martz-BIBLIOFILE uses variable-length fields with an upper limit of 500 characters which can be searched, alphabetically sorted and saved for final editing with a suitable text editing program. Each 100k of disk space holds an average

Laser exchange goes European

WHAT happens to lasers that are no longer wanted? Europe's first laser exchange offers an alternative to abandoned equipment, and to buyers who'd like to benefit from the reduced cost of a system that's already been 'broken in' (Reader Service No. 115). Multic GmbH and its Austrian sister company Physiktechnik began coordinating trade deals between laser owners and buyers last summer; since then, Multic's Peter Brandstetter says they have located new homes for over 100 lasers and laser systems. The exchange publishes a newsletter with full descriptions and prices. Transatlantic swaps can be arranged through Laser Resale, the American exchange that inspired European efforts. Commissions averaging about 10 per cent are assessed on the seller at a transaction's completion; otherwise the service is free.

of 400 references, and a single database can be split over two or more disks.

The software costs £250 (UK), and can be used with standard ASCII files in all popular word processors.

Medline and Life Sciences databases are no longer just on-line phenomena. Cambridge Scientific Abstracts has put both collections on compact optical disks for use with IBM PCs (Reader Service No. 116). The disks are updated quarterly and sold as a two-disk set, or separately. Medline, a source for biomedical literature, operates through an agreement with the US National Library of Medicine and the Department of Health and Human Services, to provide indexes for about 20,000 articles every month. The life sciences database indexes more than 98,000 abstracts each year from over 5,000 publications. A two-year subscription to both collections costs \$8,500 (US), including a \$500 charge for purchase of a CD reader. Separately, the Medline database is priced at \$6,350 with a reader, and the Life Sciences disk is \$3,650.

Methods memos

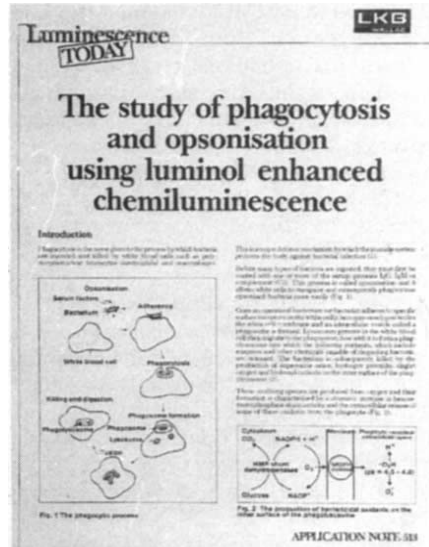
Ciba Corning Diagnostics has a forty-page brochure that describes the operation and methodology of flame photometry (Reader Service No. 117). *A Guide to Flame Photometer Analysis* outlines the apparatus, preparation and quantitation of alkali and alkaline earth metals, and describes 22 application methods including the determination of calcium in beer.

Mass detection can be the most useful type of HPLC detection for separations of polymers, resins, lipids and carbohydrates. Applied Chromatography Systems

publishes a bibliography of applications for their model 750/14 mass detector (Reader Service No. 118). ACS's detector uses the principle of separating the solvent from the sample, then distinguishing components by light scattering techniques.

To help the potential user judge the feasibility of LC/MS techniques, Kratos Analytical supplies their *LC/MS Data Compilation Book* (Reader Service No. 119). Kratos' brochure reviews the applications and accomplishments of LC/MS techniques, with attention to typical problems and solutions, and represents the broad range of research that the technique can address.

The measurement of phagocytosis by chemiluminescence has become an important technique in analysing the body's immune response. LKB Instruments discusses the principles of such measurement in their application note 513, entitled *The study of phagocytosis and opsonisation using luminol-enhanced chemiluminescence* (Reader Service No. 120). The note includes exact experimental details on cell preparation, reagent specifications and



Chemiluminescence news from LKB assay equipment. Graphic examples illustrate normal versus defective serum responses.

A new series from Whatman addresses themes in thin layer chromatography and invites contributions from readers (Reader Service No. 121). Past issues of the *Whatman Chromatography Folio* have focused on preadsorbent and high performance TLC, describing how to maximize the usefulness of these methods. The complete folio comprises a reference source for all chromatographers. □

These notes are compiled by Karen Wright from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal.

Training for competitiveness

from Richard Pearson

"The same machines and equipment can be bought by anybody; success in the market goes to those who have a workforce that can use them to best advantage."

INVESTMENT in people is being seen as the key to economic success once again. The 1960s saw the growing acceptance of the concept of 'human capital' and 'human asset accounting', and in the United Kingdom legislation set up an infrastructure to stimulate and manage training, and to redistribute its costs between the trainers through the Industrial Training Boards. By the late 1970s little progress had been made, the bureaucracies were seen to have taken over, and the incoming Conservative government enacted legislation which led to the demise of most of the training boards. In the mid-1980s training is back in vogue and the publication of *Competence and Competition* (HMSO, 1984; see *Nature* 313, 82; 1985) was part of a new campaign to boost training in the United Kingdom. It highlighted the United Kingdom's 'poor' training performance relative to West Germany, Japan and the US countries where individuals, employers and the state all placed greater value on training, seeing it as necessary capital investment, rather than as a cost.

A survey of UK employers in 1984 showed that while 90 per cent of managers recognized the importance of training, only one in three adults had received any training in the previous year. Private-sector establishments were estimated to have spent over £2,000 million on training adults that year, which averaged £200 per employee or £575 per trainee. Two-thirds of the training was done on-the-job, the rest through courses, with 4 per cent via evening classes and distance learning (Fig. 1).

While the link between training and a company's performance is hard to prove, it was shown that high-performance businesses, defined as those that were profitable, growing and innovating, were twice as likely to be training their staff as were low performers. They also trained twice as many staff and had increased their volume of training by over 25 per cent in the previous five years. By contrast, the low performers had cut their training by as much as 20 per cent over the same period.

The most recent major report in support of the national training campaign is *A Challenge to Complacency: Changing Attitudes to Training* (MSC/NEDO, 1985) which sought to identify the 'why' of our low training performance and hence the scope for encouraging companies to increase their investment in training. The report makes depressing reading. It shows

employers' lack of concern about training and highlights the widespread ignorance among top management as to the scale of resources their organization devoted to training. Decisions about training were predominantly taken by line managers who inevitably focused on short-term needs. In technical areas this is often manifest as a willingness to send people to conferences or short courses to pick up the latest technology, but a neglect or lack of

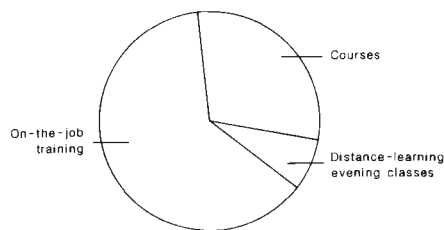


Fig. 1 Types of training. On-the-job training, courses and distance-learning/evening courses annually occupy 34 million, 17 million and 2 million days respectively. (Source: IMS)

interest in the longer-term development of staff. Little attention is paid to the analysis of training needs or evaluation of its results, and training managers and departments have relatively low status. Training is seen as a cost and overhead, to be cut when profits are under pressure, rather than as an investment in the future. In Britain, training was cut in the recession, while in West Germany and Japan the pressure was to increase the level of training given. As the remark made by a West German employer and shown in the introduction reveals, other countries are well aware that competitive advantage can only come through attuning people to a technological world.

The reluctance to invest in training was put down to uncertainties about future prospects and technological change, worries about 'poaching' by other employers and poor experiences with some external trainers. High trainee wage costs were not seen as a problem. At the same time there was little pressure from individual employees or unions, external commentators and investors, or from the government to do better, although the picture is beginning to change.

A number of proposals have been put forward to improve matters. For employers, the preparation and dissemination of case studies showing the links between training and performance are seen to be important. Employers should also be en-

couraged to report regularly on their training. This will require the development of training indicators, initially focusing on inputs such as man days, money invested (not cost!), type of training, whether it be on-the-job or off-the-job, training staff employed and so on. The process of collecting such data would be a major innovation for many companies and is likely immediately to raise the general awareness of training. In the longer term, indicators of training output or performance need to be developed but this will take some time. Individual employees can be an important stimulus for more training; in the United States, for example, it is notable the extent to which individuals seek out and invest in training. In the United Kingdom it is suggested that Individual Training Credits (ITCs) to which individuals and employers contribute, as for occupational pensions, should be set up to pay for approved training. The ITCs would need to be transferable so that they could be stored and used to retrain between jobs for example, and could also be used to incorporate grants to individuals, for example at the start of working life or following redundancy. They could also help share the costs of training more equitably and reduce the fears of losses due to poaching. Of course, if training was at a much higher level overall, there would be less poaching in the first place.

The report also argues the need to improve the operation of the training market, for example by helping colleges to become more responsive to market needs, and to improve the working of local labour markets and collaborative training arrangements. Another need is for better information on training availability; at present, many training specialists are confused at the wide variety of training on offer and find it difficult to choose the most relevant and effective course. While registers and databases help as to availability, ideally more qualitative assessments are also needed. Many other mechanisms such as grants and fiscal measures are also reviewed in the report.

The analyses of the need to train are getting more frequent, hopefully raising the awareness of its value to employee and employer alike. Time will tell if this is translated into an analysis of training needs which is then acted upon. □

Richard Pearson is at the Institute of Manpower Studies, Mantell Buildings, University of Sussex, Brighton BN1 9RF, UK.